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fibrils corresponds to 12.5 Å, which comes very close to the lattice constant to dry collagen. It is interesting to note that the orientation of the proton of the  $\gamma$ -hydroxyl group with respect to the hydroxyproline ring atoms in the above structure is very similar to that reported from the neutron diffraction studies on 4-hydroxyl-L-proline<sup>2</sup>. In fact, it is also possible to have the hydrogen bond from the Hyp OH to O<sub>3</sub> of a neighbouring microfibril through the intermediary of a water molecule. If this is made reasonably good with hydrogen bond lengths of the order of 2.75 Å, then the separation between the neighbouring microfibrils comes to 14 Å. This roughly corresponds to the intermicrofibrillar distance in collagen at normal humidities.

Thus, we see that Hyp can serve a double purpose in collagen—(a) it can form hydrogen bonds linking neighbouring peptide chains in a single triple helix and (b) it can also form hydrogen bonds with a different triple helix to link the two together. Thus, without ever forming a covalent linkage, hydroxyproline can serve a very important purpose in stabilizing the collagen structure. It may be mentioned that two recent studies have experimentally verified that the melting temperature,  $T_m$ , of collagen fibres is about 15° higher when the prolines in position 3 in the peptide chain are all hydroxylated, than when they are not hydroxylated<sup>3,4</sup>. In the work by Berg and Prockop<sup>3</sup>, the  $T_m$  was measured directly from optical rotation studies and

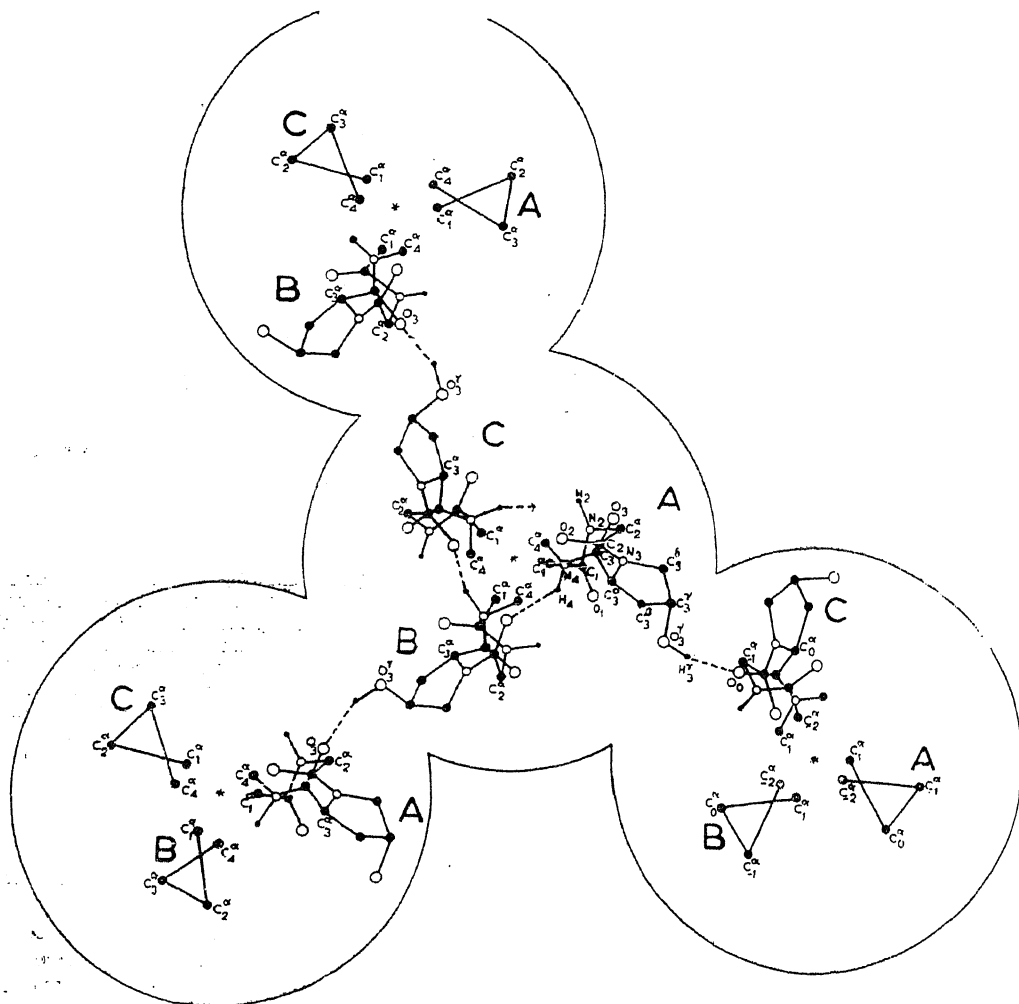


FIG. 2. The hydrogen bond scheme linking O $\gamma$ H $\gamma$  of one microfibril in position 3 with O<sub>3</sub> of the next microfibril is shown for a central triple helix along with the three neighbouring microfibrils in hexagonal directions. It is to be noted that this linkage can be continued to form a good hexagonal lattice for the structure.

was shown to be appreciably larger for the hydroxylated form of collagen than the unhydroxylated form from the same source. In the latter studies by Jimenez *et al.*<sup>4</sup>, the thermal stability of unhydroxylated collagen relative to hydroxylated collagen was investigated using pepsin digestion at various temperatures as an enzymatic probe of conformation. Their results also indicate that the unhydroxylated molecules have a denaturation temperature between 20° and 25°, while the hydroxylated molecules are stable beyond 35°. These studies can be taken to be very good evidence in support of the theoretical ideas put forward from our laboratory regarding the role played by hydroxyproline in the stability of the collagen molecule.

This work was supported by U.S. PHS Grants AM-15964 in Bangalore and AM-11493 in Chicago. M. B. is grateful to the CSIR, India, for a scholarship.

1. Ramachandran, G. N., Bansal, M. and Bhatnagar, R. S., *Biochim. Biophys. Acta*, 1973, 322, 166.
2. Koetzie, T. F., Lehmann, M. S. and Hamilton, W. C., *Acta Cryst.*, 1973, 29 B, 231.
3. Berg, R. A. and Prockop, D. J., *Biochem. Biophys. Res. Commun.*, 1973, 52, 115.
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## AMPEROMETRIC, POTENTIOMETRIC AND MAGNETIC STUDIES OF SOME RARE EARTH KOJATES

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### ABSTRACT

The reactions of the rare earth elements ( $\text{La}^{+3}$ ,  $\text{Ce}^{+3}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$  and  $\text{Gd}^{+3}$ ) with kojic acid have been studied by employing amperometric and potentiometric titrations. The results of these titrations reveal 1:3 (metal:ligand) stoichiometry for the complexes. The experimental magnetic moments are in agreement with those reported for typical lanthanide sulphates and other compounds, indicating that metal ion in these chelates acts as a free ion, as far as the *f*-electrons are concerned.

### INTRODUCTION

**R**ARE earth complexes of kojic acid of the general composition  $[\text{M}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$  (where  $\text{M} = \text{La}^{+3}$ ,  $\text{Ce}^{+3}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$ ,  $\text{Gd}^{+3}$ ,  $\text{Dy}^{+3}$ ,  $\text{Ho}^{+3}$  and  $\text{Y}^{+3}$ ) were prepared recently by us<sup>1-3</sup> and characterised. Analytical data and IR spectra of complexes show kojic acid acting as bidentate ligand and two water molecules are also present in the coordination sphere. In this communication the reactions of rare earth elements ( $\text{La}^{+3}$ ,  $\text{Ce}^{+3}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$  and  $\text{Gd}^{+3}$ ) have been studied by employing amperometric and potentiometric titrations. Magnetic susceptibility data on the complexes are also reported in this paper.

### MATERIALS AND METHODS

All chemicals were of BDH (AR) quality. Elico model LI-10 pH meter, in conjunction with a glass electrode and a SCE, was used for potentiometric titrations conducted at 30°C. Amperometric and magnetic measurements were made as reported in the earlier communication<sup>2</sup>.

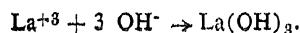
### RESULTS AND DISCUSSION

**Amperometric titrations.**—The amperometric titrations, both direct and reverse, were carried out, using 2 M sodium nitrate as supporting electrolyte and 0.2% gelatine solution as maximum suppressor, at the plateau of the rare earth ion (− 1.2 V) and the ligand (− 0.95 V) respectively.

From the results of these titrations it is indicated that the mole ratio in which the metal and the ligand combine to form the respective complex is 1 : 3.

**Potentiometric titrations.**—The titrations were carried out with the solutions containing the metal and the ligand in the ratios of 1:0, 1:1, 1:2 and 1:3 against 0.1 M NaOH.

The solution containing lanthanum nitrate alone exhibits only one inflexion point (Fig. 1, curve A), corresponding to the interaction of 3 moles of NaOH with 1 mole of lanthanum to give 1 mole of lanthanum hydroxide.



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# HYDROXYPROLINE STABILIZES BOTH INTRAFIBRILLAR STRUCTURE AS WELL AS INTER-PROTOFIBRILLAR LINKAGES IN COLLAGEN\*

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## ABSTRACT

It is shown that the previous report from the author's laboratory that hydroxyproline has a role in stabilizing collagen structure can be slightly modified to make it serve the purpose of not only leading to hydrogen bonds between two chains in the triple helix, but also in linking one triple helix with another. The modified scheme of hydrogen bonding is discussed and illustrated with diagrams.

IN a recent paper<sup>1</sup>, it was reported from our laboratory that hydroxyproline can play an important part in stabilizing the collagen structure by forming hydrogen bonds, with its hydroxyl group ( $O^{\gamma}H^{\gamma}$ ) playing a vital role in this linkage. The essence of the type of hydrogen-bonded linkages that was proposed is continued in the diamond shaped region shown in Fig. 1 of that paper. A more careful re-examination of this structure indicates that it would be possible for the hydroxyproline OH to be involved both in a hydrogen bond connecting two neighbouring chains (say A and B) in the triple helix, as well as to form a hydrogen bond with a neighbouring triple-helical protofibril. This arrangement of hydrogen bonds is shown in Fig. 1, where only the relevant atoms of chain A and B are shown in detail. It will be seen that the NH group ( $N_2H_2$ ), corresponding to the second residue in the sequence  $-Gly-X-Y-$  which occurs in collagen, forms a hydrogen bond with  $O_1$  of a neighbouring chain via the water molecule  $O^w$  ( $H_1^w, H_2^w$ ). Of the two protons in the water, one forms an almost straight hydrogen bond with  $O_1$  of chain A, while the second proton forms a hydrogen bond with the  $O_3^{\gamma}$  atom of chain A acting as a receptor for this bond (the standard nomenclature of referring to all atoms belonging to the same residue by the same subscript is adopted in this paper, which is slightly different from the earlier notation of denoting all atoms in the same peptide unit by the same subscript, which was adopted in Ref. 1). It will be seen, therefore, that the water molecule does not have any free proton available for external linkage in this model. For

the same reason, the water medium in the structure cannot easily disturb the water proton by forming a hydrogen bond via that atom.

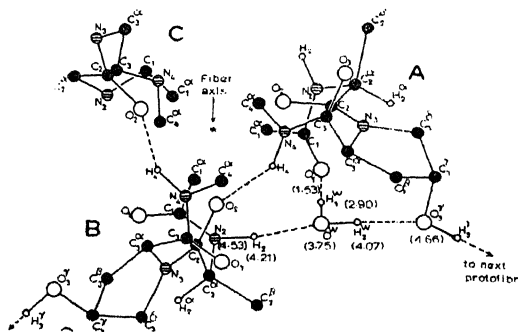


FIG. 1. Diagram showing the hydrogen bonding arrangement in a protofibril of collagen involving the hydroxyproline OH group and a water molecule. The water molecule is linked differently with the Hyp  $O^{\gamma}$  from what was previously reported in Ref. 1. Here the water donates a proton to  $O^{\gamma}$  and the  $O^{\gamma}$  proton is the donor of the hydrogen bond linking the protofibril chain to a neighbouring protofibril. (The numbers denote the heights of the atoms concerned parallel to the fibre axis.)

What is more interesting is the fact that the proton  $H_3^{\gamma}$  of the hydroxyl group in residue 3 of chain A is now available for linking one protofibril with another. This is shown in Fig. 2 in which the linking hydrogen bond from Hyp for a central protofibril is shown with three other protofibrils in outline in the neighbourhood of this. The diagram shown corresponds to the case in which the hydrogen bond is direct between  $O_3^{\gamma}H_3^{\gamma}$  of the central protofibril and the  $O_3$  of the neighbouring protofibril. The distance between the two proto-

\* Contribution No. 53 from the Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560 012, India.

fibrils corresponds to 12.5 Å, which comes very close to the lattice constant to dry collagen. It is interesting to note that the orientation of the proton of the  $\gamma$ -hydroxyl group with respect to the hydroxyproline ring atoms in the above structure is very similar to that reported from the neutron diffraction studies on 4-hydroxyl-L-proline<sup>2</sup>. In fact, it is also possible to have the hydrogen bond from the Hyp OH to  $O_{\beta}$  of a neighbouring protofibril through the intermediary of a water molecule. If this is made reasonably good with hydrogen bond lengths of the order of 2.75 Å, then the separation between the neighbouring protofibrils comes to 14 Å. This roughly corresponds to the interprotofibrillar distance in collagen at normal humidities.

Thus, we see that Hyp can serve a double purpose in collagen—(a) it can form hydrogen bonds linking neighbouring peptide chains in a single triple helix and (b) it can also form hydrogen bonds with a different triple helix to link the two together. Thus, without ever forming a covalent linkage, hydroxyproline can serve a very important purpose in stabilizing the collagen structure. It may be mentioned that two recent studies have experimentally verified that the melting temperature,  $T_m$ , of collagen fibres is about 15° higher when the prolines in position 3 in the peptide chain are all hydroxylated, than when they are not hydroxylated<sup>3,4</sup>. In the work by Berg and Prockop<sup>3</sup>, the  $T_m$  was measured directly from optical rotation studies and

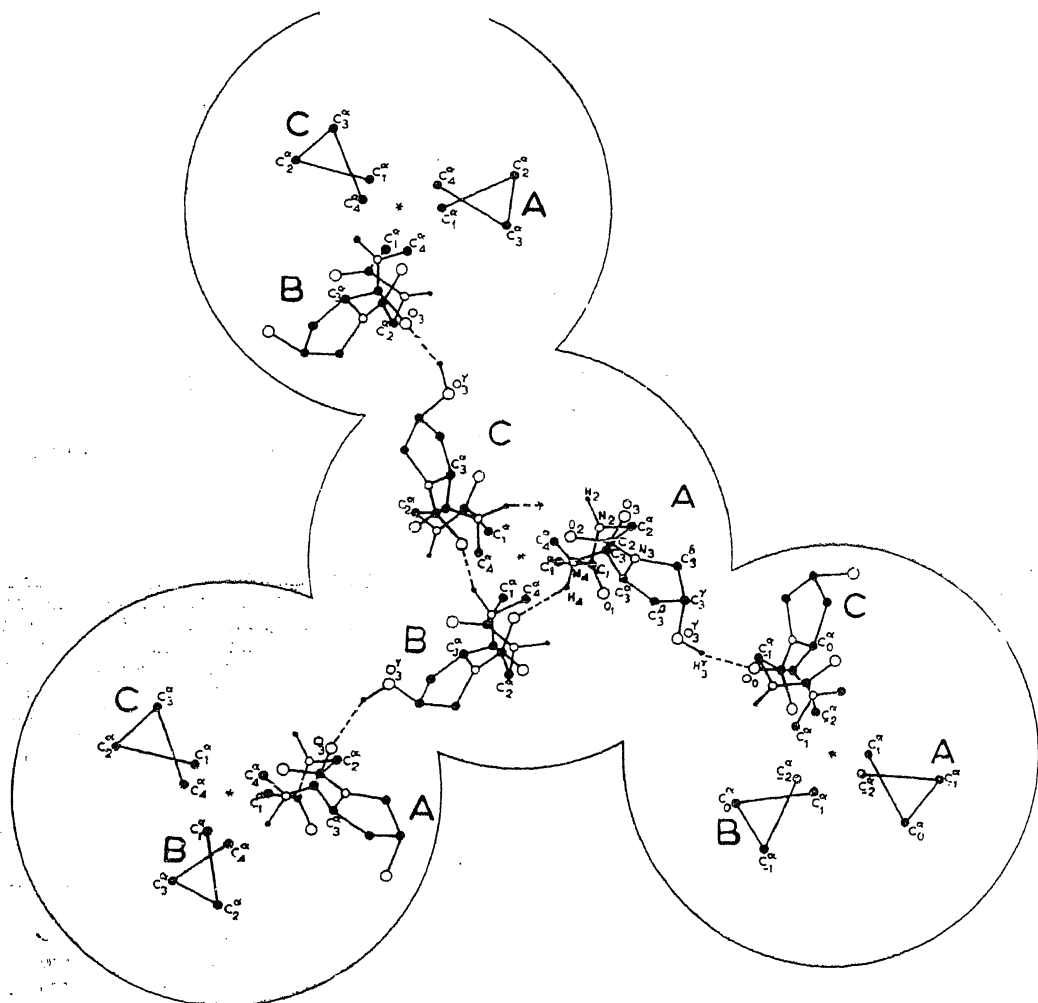


FIG. 2. The hydrogen bond scheme linking  $O\gamma H\gamma$  of one protofibril in position 3 with  $O_{\beta}$  of the next protofibril is shown for a central triple helix along with the three neighbouring protofibrils in hexagonal directions. It is to be noted that this linkage can be continued to form a good hexagonal lattice for the structure.

On the addition of 1 mole of the ligand to the lanthanum nitrate solution, the pH of the solution is lowered (Fig. 1, curve B). This lowering of pH can be attributed to the release of protons during complex formation. The curve shows two inflexion points, one corresponding to 1 mole and the other to 3 moles of NaOH respectively. As the solution contains lanthanum and the ligand in 1:1 ratio, it is assumed that 1/3 of the metal reacts with the ligand to form 1:3 complex with the release of a proton which is neutralized by 1 mole of NaOH, while the remaining 2/3 of the metal is precipitated as hydroxide on the addition of another 2 moles of NaOH.

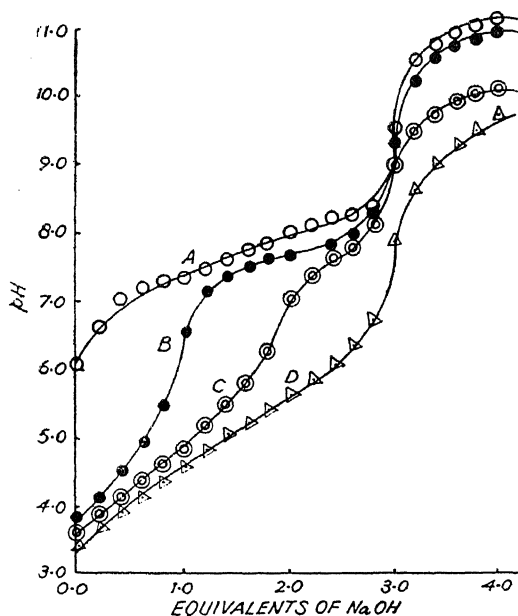


FIG. 1. pH-metric titration curves. A, 20 ml 0.005 M lanthanum nitrate + 30 ml  $H_2O$ ; B, 20 ml 0.005 M lanthanum nitrate + 5 ml 0.02 M kojic acid + 25 ml  $H_2O$ ; C, 20 ml 0.005 M lanthanum nitrate + 10 ml 0.02 M kojic acid + 20 ml  $H_2O$ ; D, 20 ml 0.005 M lanthanum nitrate + 15 ml 0.02 M kojic acid + 15 ml  $H_2O$ ; titrant, 0.1 M NaOH.

The titration of the solution containing metal and the ligand in 1:2 ratio (Fig. 1, curve C) shows two inflexion points, one corresponding to 2 moles and the other to 3 moles of NaOH respectively. This clearly indicates that at 2 equivalence the 2 protons were neutralized and other one equivalence of NaOH is required for the hydroxide formation.

Similarly with the solution containing metal and the ligand in 1:3 ratio (Fig. 1, curve D) shows only one inflexion point corresponding to 3 moles of NaOH, which are required to neutralize the 3 protons released during 1:3 complex formation.

Similar potentiometric behaviour was observed with the other rare earth ions ( $Ce^{+3}$ ,  $Pr^{+3}$ ,  $Nd^{+3}$ ,  $Sm^{+3}$  and  $Gd^{+3}$ ) showing thereby the existence of 1:3 complexes in these systems also.

**Magnetic measurements.**—Results of magnetic measurements at room temperature are presented in Table I. The effective magnetic moment has been calculated from corrected molar susceptibilities by Curie law.

$$\mu_{eff} = 2.84 \sqrt{\chi_M (\text{corr.})^T}$$

TABLE I

Magnetic susceptibilities of rare earth kojates

| Complex                                      | Temp. $\times 10^6$<br>° K | $\chi' \times 10^6$ | $\chi'_M \times 10^6$ | $\mu_{eff}$<br>(BM) |
|--|----------------------------|---------------------|-----------------------|---------------------|
| [Ce ( $C_6H_5O_4$ ) <sub>3</sub> ( $H_2O$ )] | 303.0                      | 4.04                | 2020                  | 2.33                |
| [Pr ( $C_6H_5O_4$ ) <sub>3</sub> ( $H_2O$ )] | 303.0                      | 10.18               | 5907                  | 3.69                |
| [Nd ( $C_6H_5O_4$ ) <sub>3</sub> ( $H_2O$ )] | 302.5                      | 8.08                | 4908                  | 3.46                |
| [Sm ( $C_6H_5O_4$ ) <sub>3</sub> ( $H_2O$ )] | 302.5                      | 1.95                | 987                   | 1.53                |
| [Gd ( $C_6H_5O_4$ ) <sub>3</sub> ( $H_2O$ )] | 302.5                      | 43.14               | 26390                 | 7.90                |

$\chi =$  Magnetic susceptibility per g of material,

$\chi'_M =$  Corrected magnetic susceptibility per g mole.

The  $La^{+3}$  chelate under examination was found to be diamagnetic. The magnetic moments obtained experimentally for the remaining rare earth chelates are in good agreement with the values for typical lanthanide sulphates<sup>4</sup>. These values suggest that the lanthanide ion acts approximately as free ion, as far as the *f*-electrons are concerned.

## ACKNOWLEDGEMENT

The authors are indebted to Dr. W. U. Malik, Professor and Head, Chemistry Department, Roorkee University, Roorkee, for helpful discussions, criticism and constant encouragement.

1. Agarwala, R. C., Gupta, S. P. and Rastogi, D. K., *J. Inorg. Nucl. Chem.*, 1974, 36, 208.
2. — and —, *Curr. Sci.*, 1974, 43, 263.
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was shown to be appreciably larger for the hydroxylated form of collagen than the unhydroxylated form from the same source. In the latter studies by Jiminez *et al.*<sup>4</sup>, the thermal stability of unhydroxylated collagen relative to hydroxylated collagen was investigated using pepsin digestion at various temperatures as an enzymatic probe of conformation. Their results also indicate that the unhydroxylated molecules have a denaturation temperature between 20° and 25°, while the hydroxylated molecules are stable beyond 35°. These studies can be taken to be very good evidence in support of the theoretical ideas put forward from our laboratory regarding the role played by hydroxyproline in the stability of the collagen molecule.

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## AMPEROMETRIC, POTENTIOMETRIC AND MAGNETIC STUDIES OF SOME RARE EARTH KOJATES

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### INTRODUCTION

RARE earth complexes of kojic acid of the general composition  $[\text{M}(\text{C}_6\text{H}_7\text{O}_4)_3(\text{H}_2\text{O})_2]$  (where  $\text{M} = \text{La}^{+3}$ ,  $\text{Ce}^{+3}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$ ,  $\text{Gd}^{+3}$ ,  $\text{Dy}^{+3}$ ,  $\text{Ho}^{+3}$  and  $\text{Y}^{+3}$ ) were prepared recently by us<sup>1,2</sup> and characterised. Analytical data and IR spectra of complexes show kojic acid acting as bidentate ligand and two water molecules are also present in the coordination sphere. In this communication the reactions of rare earth elements ( $\text{La}^{+3}$ ,  $\text{Ce}^{+3}$ ,  $\text{Pr}^{+3}$ ,  $\text{Nd}^{+3}$ ,  $\text{Sm}^{+3}$  and  $\text{Gd}^{+3}$ ) have been studied by employing amperometric and potentiometric titrations. Magnetic susceptibility data on the complexes are also reported in this paper.

### MATERIALS AND METHODS

All chemicals were of BDH (AR) quality. Elico model LI-10 pH meter, in conjunction with a glass electrode and a SCE, was used for potentiometric titrations conducted at 30° C. Amperometric and magnetic measurements were made as reported in the earlier communication<sup>2</sup>.

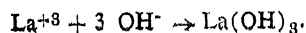
### RESULTS AND DISCUSSION

**Amperometric titrations.**—The amperometric titrations, both direct and reverse, were carried out, using 2 M sodium nitrate as supporting electrolyte and 0.2% gelatine solution as maximum suppressor, at the plateau of the rare earth ion (— 1.2 V) and the ligand (— 0.95 V) respectively.

From the results of these titrations it is indicated that the mole ratio in which the metal and the ligand combine to form the respective complex is 1:3.

**Potentiometric titrations.**—The titrations were carried out with the solutions containing the metal and the ligand in the ratios of 1:0, 1:1, 1:2 and 1:3 against 0.1 M NaOH.

The solution containing lanthanum nitrate alone exhibits only one inflexion point (Fig. 1, curve A), corresponding to the interaction of 3 moles of NaOH with 1 mole of lanthanum to give 1 mole of lanthanum hydroxide.



# OXIDATION OF CYSTEINE WITH CHLORAMINE-T AND DICHLORAMINE-T

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THE oxidation of cysteine and other thiols<sup>1</sup> has been the subject of a number of investigations. The sulphhydryl group is generally oxidized to the corresponding disulphide, although instances of oxidation beyond the disulphide stage are known<sup>2,3</sup>. Kinetic investigations of the oxidation of thiols as a rule require rapid analytical techniques. Cysteine is an important amino acid and several analytical reagents<sup>4-11</sup> have been employed for assaying this compound by direct or through instrumental methods. In the present investigations, we have examined the behaviour of chloramine-T (CAT) and dichloramine-T (DCT) towards cysteine and we are reporting about some simple analytical methods devised for estimating the compound, with these reagents.

**Materials.**—L(+)-Cysteine (E. Merck) was purified by recrystallization from aqueous solution and was assayed to 95.5% by the iodimetric method<sup>4</sup>. The purity of the sample was checked by TLC and paper chromatographic methods, where it gave a single spot. An aqueous solution (~ 2 mg per ml) was prepared by dissolving the compound in water containing a few drops of dilute HCl. Other solutions of cysteine were prepared by dissolving the solid in appropriate buffers<sup>12</sup> and solvents.

CAT (E. Merck) was purified by the method of Morris *et al.*<sup>13</sup>. An approximately decinormal solution was prepared and standardized by the iodometric method. DCT was prepared and standardized by the method of Jacob and Nair<sup>14</sup>. Reagent grade materials were used in preparing solutions of other compounds. All solutions were prepared in triple distilled water.

Two methods were adopted for estimating the thiol.

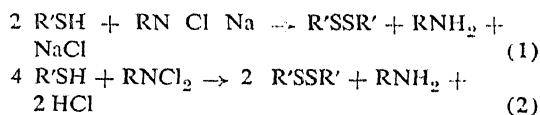
(I) *Direct Titration Procedure*: Preliminary experiments showed that aqueous cysteine solutions can be directly titrated against CAT with starch-KI internal indicator in presence of dilute H<sub>2</sub>SO<sub>4</sub>. The overall concentration of H<sub>2</sub>SO<sub>4</sub> should be maintained at least at 0.2 N and higher acid concentrations did not affect the results. In the recommended procedure, aliquots of aqueous cysteine solution are taken in a titration flask. About 2 ml of starch-KI mixture and enough of 2 N H<sub>2</sub>SO<sub>4</sub> to make the overall concentration 0.2 N

are added. The solution is titrated against standard CAT solution to the appearance of a pale blue colour. The values are found to be reproducible.

A potentiometric titration between aqueous cysteine and CAT solutions was found to be unsuitable as no sharp potential break could be observed under the experimental conditions.

A direct titration of cysteine in glacial acetic acid solution against a 0.02192 N solution of DCT (in glacial acetic acid) was carried out potentiometrically and by visual end-point method as described for thoglycolic acid<sup>15</sup>. A potential break of about 100 mv was recorded for a 0.1 ml addition of titrant near the end-point.

Oxidation of the thiol involves a single electron change with both CAT and DCT, which can be stoichiometrically represented as:



where R' = HOOC.CH(NH<sub>2</sub>).CH<sub>2</sub> and R = p-CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>. The presence of disulphide (R<sub>2</sub> = 0.044) was detected by paper chromatography with butanol-acetic acid-water (4:1:5 v/v) as solvent and ninhydrin spray reagent (0.2% solution in butanol-water-acetic acid 95:4:0.5 v/v).

Some typical results of analyses are given in Table I. It can be seen that CAT and DCT can be used for a rapid and accurate assay of thiol.

The interference of some amino acids and related compounds in the estimation of cysteine was investigated. Lysine, leucine, glutamine, methionine and urea (~ 0.1 mmole) did not interfere in the estimation with CAT, while histidine, alanine, valine, serine, threonine, arginine, glycine, proline and thiourea interfered. Although a similar behaviour was noticed with DCT titrations, leucine and glutamine interfered while arginine, glycine, proline and urea had no effect.

(II) *Back Titration Procedure*: In preliminary experiments with CAT, known amounts of cysteine solution (~ 10 mg) prepared in the appropriate buffer were added to a known excess volume of CAT solution (~ 1.25 mmoles) in an iodine flask. The reaction mixture was set aside for various intervals of time, with occasional shaking. Then the excess CAT left unconsumed was determined

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TABLE I  
Oxidation of cysteine with chloramine-T and dichloramine-T

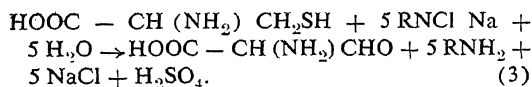
| Titrant CAT      |          | Titrant DCT      |          |                          |          | Titration CAT         |          |
|------------------|----------|------------------|----------|--------------------------|----------|-----------------------|----------|
| Direct titration |          | Direct titration |          | Potentiometric titration |          | Back titration method |          |
| Weight of thiol  |          | Weight of thiol  |          | Weight of thiol          |          | Weight of thiol       |          |
| Taken mg         | Found mg | Taken mg         | Found mg | Taken mg                 | Found mg | Taken mg              | Found mg |
| 9.26             | 9.26     | 9.92             | 9.96     | 10.04                    | 10.09    | 5.96                  | 6.01     |
| 18.52            | 18.51    | 19.83            | 20.18    | 20.07                    | 20.19    | 9.93                  | 9.92     |
| 27.78            | 27.62    | 34.70            | 34.80    | 30.11                    | 29.89    | 19.86                 | 19.91    |
| 37.04            | 36.88    | 44.61            | 44.61    | 40.15                    | 40.12    | 23.84                 | 23.69    |
| 57.23            | 57.51    | 49.56            | 49.66    | 50.19                    | 50.73    | 29.80                 | 29.55    |
| 76.31            | 76.31    | 59.48            | 59.60    | 60.22                    | 60.28    | 33.77                 | 33.67    |
| 95.39            | 95.96    | 69.39            | 69.46    | ..                       | ..       | 36.60                 | 36.61    |

by back titration. A comparison of the extent of oxidation after 30 min. showed that there is a 10 electron change per mole of cysteine in buffer media of pH 1-3, which decreases with the increase in pH, i.e., 9.5 at pH 5, 8.8 at pH 7 and 6.3 in 0.1 N NaOH. The results were quite reproducible in the pH range 1-3 and the following procedure was therefore used for estimating the thiol. A solution of cysteine in pH 1 buffer (~2 mg/ml) was prepared. Aliquots of the solution were added to 50 ml of decinormal CAT solution in an iodine flask. The mixture was shaken occasionally and after 30 min. 10 ml of 2 N  $H_2SO_4$  and 10 ml of 20% KI were added and the liberated iodine was titrated against standard thiosulphate. The amount ( $x$  mg) of cysteine in the experimental solution is given by

$$x = 12.12 y (v_1 - v_2),$$

where  $y$  is the normality,  $v_1$  is the blank titration and  $v_2$  the volume of thiosulphate used to titrate the excess of CAT after oxidation of cysteine.

Stoichiometry of the above oxidation could probably be represented as follows:



Paper chromatography was used to identify the reaction products. Benzyl alcohol saturated with water was used as the solvent for detecting the sulphonamide ( $R_f = 0.905$ ) and 0.5% vanillin in 1% HCl solution in ethanol was the spray reagent. Attempts were made to detect the aldehydic amino acid with the amino acid solvent and spray reagent, employed for the disulphide. A spot corresponding to  $R_f = 0.091$  was observed and this probably can be taken as evidence for the compound.

Typical results of analyses are shown in Table I. The back titration procedure with DCT was unsatisfactory as the reaction was sluggish and showed only a 5-6 electron change per mole of cysteine (in glacial acetic acid solution) even after 40 minutes.

One of us (D. S. M.) acknowledges financial assistance from the University Grants Commission, New Delhi, India.

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## LETTERS TO THE EDITOR

### VARIATION OF INTERNAL PRESSURE OF MERCURY WITH APPLIED PRESSURE AND TEMPERATURE

THE importance of the measurement of internal pressure of pure liquids, mixture of liquids and solutions has been stressed<sup>1-3</sup>. In this communication, the variation of the internal pressure of mercury, with applied pressure is studied, using the experimentally obtained data<sup>1</sup> for the computation.

*Method of Calculation of the Internal Pressure ( $\pi$ ).*

$$\pi = \left( \frac{\partial E}{\partial V} \right)_T - T \left( \frac{\partial S}{\partial V} \right)_T - p \quad (1)$$

From the thermodynamic equation of state.

$$\left( \frac{\partial S}{\partial V} \right)_T = \left( \frac{\partial p}{\partial T} \right)_V \quad (2)$$

From Maxwell's thermodynamic relations.

$$\left( \frac{\partial p}{\partial T} \right)_V = \left[ \frac{\partial p}{\partial V} \right]_T \left[ \frac{1}{V} \frac{\partial V}{\partial T} \right]_p \quad (3)$$

Therefore, equation (1) can be written as

$$\pi = \frac{T\alpha}{\beta_T} - p \quad (4)$$

where  $\alpha$  is the isobaric volume expansivity,  $\beta_T$  the isothermal compressibility,  $T$  the absolute temperature and  $p$  the pressure at which the system is examined.

$$\alpha = \frac{V_2 - V_1}{V_1(T_2 - T_1)} \quad (5)$$

where  $V_1$  and  $V_2$  are the volumes at  $T_1^\circ$  K and  $T_2^\circ$  K respectively, the volumes being measured at constant pressure.

$$\beta_T = \frac{V_2' - V_1'}{V_1'(P_2 - P_1)} \quad (6)$$

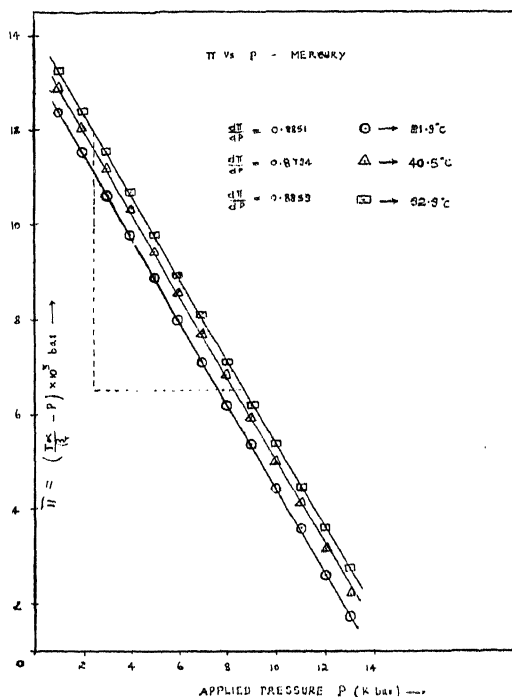
where  $V_1'$  and  $V_2'$  are the volumes at pressures  $P_1$  and  $P_2$  respectively when the temperature of the system is constant.

**Results and Discussion.**—The graph presented gives the variation of internal pressure with applied pressure at three different temperatures. Since it is linear with a negative slope, one can expect the internal pressure to pass through zero and become negative if the external pressure is increased. The internal pressure is due to intermolecular forces. As the applied pressure increases the repulsive forces between the molecules become dominant and this decreases the internal pressure. It is seen that the slope is almost the same at the temperatures at which the system is examined.

It is also observed that the internal pressure increases with the temperature when the pressure

is maintained constant. As the temperature is increased the distance between the conduction electrons in mercury increases which decreases the repulsive forces. Hence there is an increase in the internal pressure as the temperature is increased.

This study brings out the role of the repulsive forces in the internal pressure of liquids. Further study of the internal pressure in molten metals is in progress.



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# ANALYSIS OF THE PHENOMENON OF MISCIBILITY IN A TERNARY SYSTEM: WATER-CARBON TETRACHLORIDE-METHANOL

TERNARY systems, in which a third component added to a mixture of two immiscible components brings about the disappearance of the phase boundary, resulting in a homogenous mixture, have been studied earlier by Suryanarayana and Somasundaram<sup>1,2</sup> from the point of view of dielectric contribution to miscibility. Recently we have studied the system water-carbon tetrachloride-methanol from the point of view of ultrasonics and other properties. We report herein an analysis of the phenomenon of miscibility.

*Experiments.*—All the components have been purified as described earlier<sup>3</sup>.

Equal volumes of water and carbon tetrachloride (100 ml each) were taken in a clean conical flask and kept in a thermostat maintained at  $35^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ . A known quantity of methanol was added, stirred well and allowed to attain equilibrium. Then the two layers were separated and the physico-chemical properties measured for each layer.

Ultrasonic velocity, density, dielectric constant and refractive index have been determined as given earlier<sup>3</sup>.

*Results and Discussion.*—It is expected normally that the added methanol will distribute between water and carbon tetrachloride. What is really happening is that as more and more methanol is added more carbon tetrachloride is being extracted into the top layer (Column 4 of Table II). An examination of the physico-chemical properties of the bottom layer indicates the presence of a single component (carbon tetrachloride), irrespective of the amount of methanol added. Bonner<sup>4</sup> made a similar observation on the system ether-water-ethanol. The added third component seems to modify the properties of only one layer and the binary mixture progressively extracts the second immiscible layer into it.

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TABLE I  
*Physico-chemical properties of pure solvents at  $35^{\circ}\text{C}$*

| Solvent              | Density<br>(g/cc)<br>$\rho$ | Ultrasonic<br>velocity<br>(m/s)<br>$u$ | Adiabatic<br>compressibility<br>$\beta_{ad} \times 10^{12}$<br>( $\text{cm}^2 \text{dyne}^{-1}$ ) | Refractive<br>index<br>$\mu$ | Dielectric constant at |        |        |        |
|----------------------|-----------------------------|--|---|------------------------------|------------------------|--------|--------|--------|
|                      |                             |  |   |                              | 1 Mc/s                 | 2 Mc/s | 3 Mc/s | 4 Mc/s |
| Water                | 0.994                       | 1469                                   | 46.5  | 1.322                        | 78.0                   | ..     | ..     | ..     |
| Methanol             | 0.786                       | 1051                                   | 116.3   | 1.332                        | 40.3                   | 36.3   | 33.8   | 33.3   |
| Carbon tetrachloride | 1.568                       | 849                                    | 88.4  | 1.460                        | 2.2                    | 2.2    | 2.2    | 2.2    |

TABLE II  
*Physico-chemical properties of the two layers at  $35^{\circ}\text{C}$  after distribution of methanol*

| Top layers  |                               |                            |   |                       |                        |           |           |           |
|---|-------------------------------|----------------------------|---|-----------------------|------------------------|-----------|-----------|-----------|
| Vol. (ml) of methanol<br>added to 100 ml $\text{H}_2\text{O}$<br>plus 100 ml $\text{CCl}_4$ | Density<br>( $\rho$ )<br>g/cc | U.S.<br>vel.<br>$u$<br>m/s | Adi. comp.<br>$\beta_{ad} \times 10^{12}$<br>$\text{cm}^2 \text{dyne}^{-1}$ | Ref.<br>Ind.<br>$\mu$ | Dielectric constant at |           |           |           |
|   |                               |                            |   |                       | 1<br>Mc/s              | 2<br>Mc/s | 3<br>Mc/s | 4<br>Mc/s |
| 50  | 0.948                         | 1481                       | 48.1  | 1.344                 | 79.00                  | 71.25     | 66.75     | 67.75     |
| 100   | 0.923                         | 1436                       | 52.6  | 1.345                 | 71.75                  | 62.00     | 57.75     | 57.50     |
| 200   | 0.854                         | 1356                       | 63.7  | 1.345                 | 61.75                  | 58.75     | 53.00     | 52.50     |
| 300   | 0.910                         | 1215                       | 77.5  | 1.348                 | ..                     | ..        | ..        | ..        |
| Bottom layer  |                               |                            |   |                       |                        |           |           |           |
| Vol. (ml) of Methanol<br>added to 100 ml $\text{H}_2\text{O}$<br>plus 100 ml $\text{CCl}_4$ | Density<br>( $\rho$ )<br>g/cc | U.S.<br>vel.<br>$u$<br>m/s | Adi. comp.<br>$\beta_{ad} \times 10^{12}$<br>$\text{cm}^2 \text{dyne}^{-1}$ | Ref.<br>Ind.<br>$\mu$ | Dielectric constant at |           |           |           |
|   |                               |                            |   |                       | 1<br>Mc/s              | 2<br>Mc/s | 3<br>Mc/s | 4<br>Mc/s |
| 50  | 1.557                         | 850.6                      | 88.7  | 1.459                 | 2.2                    | 2.2       | 2.2       | 2.2       |
| 100   | 1.550                         | 842.6                      | 90.9  | 1.459                 | 2.26                   | 2.24      | 2.25      | 2.24      |
| 200   | 1.478                         | 853.3                      | 92.9  | 1.460                 | 2.26                   | 2.25      | 2.26      | 2.26      |
| 300   | 1.545                         | 866                        | 86.3  | 1.459                 | ..                     | ..        | ..        | ..        |

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### EVALUATION OF AVERAGE L-SHELL FLUORESCENCE YIELDS INVOLVING PHOTO-EXCITATION

THE extensive data on average L-shell fluorescence yields ( $\bar{\omega}_L$ ) using direct fluorescent excitation for creating primary vacancies, that are available at present, dates back to 1934<sup>1</sup>. Since then more reliable data on L subshell photoelectric absorption, L subshell fluorescence yields and Coster-Kronig yields, needed for the evaluation of  $\bar{\omega}_L$ , have been made available in literature. We have evaluated  $\bar{\omega}_L$  for some elements in the range Z varying from 65 to 96 by using Scofield's<sup>2</sup> data of 1973 for the determination of primary vacancy distribution among L subshells involving photo-excitation at 20 keV (except for Cm) and 200 keV and most recent values of L subshells fluorescence yields and Coster-Kronig yields as recommended in a recent review article<sup>3</sup> of 1972. The values at 20 keV agree with those at 200 keV within errors involved because of the uncertainties in the experimental data on L subshell fluorescence yields and Coster-Kronig yields, showing that  $\bar{\omega}_L$  does not depend very much upon the energy of exciting radiations. Our values for  $\bar{\omega}_L$  at 20 keV are compared with those of Lay obtained in 1934 at 17.443 keV (Energy of Mo K $\alpha$  X-rays) in Table 1. It is seen that the values of Lay for all elements except W are at least 10% higher than the present values. Reliable data on the fluorescence yields are needed because of their many applications in a large variety of measure-

TABLE I  
The present values of average L shell fluorescence yields involving photoelectric excitation are compared with the existing data

| Z  | Element | Average L shell fluorescence yield |                                |
|----|---------|------------------------------------|--------------------------------|
|    |         | Present value                      | Existing data Lay <sup>1</sup> |
| 65 | Tb      | 0.218                              | ..                             |
| 73 | Ta      | 0.248                              | ..                             |
| 74 | W       | 0.264                              | 0.298                          |
| 78 | Pt      | 0.297                              | 0.348                          |
| 79 | Au      | 0.306                              | 0.365                          |
| 81 | Tl      | 0.321                              | ..                             |
| 82 | Pb      | 0.344                              | 0.398                          |
| 96 | Cm      | 0.635                              | ..                             |

ments in the fields of Atomic and Nuclear Physics as outlined by Bambynek *et al.*<sup>3</sup>.

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### ASCORBIC ACID OXIDASE IN THE RIPENING OF BANANAS

THE most significant factor responsible for the formation of dehydroascorbic acid in plant extract is the role of ascorbic acid oxidase. Thornton<sup>1</sup> reported that fruit contained an enzyme capable of destroying ascorbic acid. The present study reports the variation of ascorbic acid oxidase during the ripening of different varieties of banana, viz., Basrai, Harichal, Lalkel (variety of *Musa cavendishii*), Rajeli, Safed velchi (variety of *Musa paradisiaca*) at 13° C.

Basrai banana was obtained from Jalgaon District, Maharashtra State, while other varieties of bananas were obtained from Bassein Road, Bombay. In order to get banana bunches of uniform maturity, nearly 100 banana plants were tagged at the time of inflorescence emergence in a nearby banana plantation where uniform cultural practices were maintained throughout the growing season. From the above lots, two bunches each of uniform development were harvested at 100 days of growth after the inflorescence emergence. Harvesting period from inflorescence emergence was almost the same in all varieties. These bunches were immediately brought to the laboratory, separated into hands and upper hands of the bunches were stored in an

incubator at a temperature of 13° C. The ratio between pulp to skin weight was determined to assess the maturity of the fruits. In our previous experiment, 13° C was found to be more favourable temperature for ripening, and storage of bananas. Therefore the enzyme activity was carried out at this temperature. All the observations were made on detached banana fingers. Three fingers from each of the five banana hands were removed at a time and the average values are reported.

In order to find out the solubility of ascorbic acid oxidase, water, 3% sodium chloride, 30% alcohol and phosphate buffer (pH 7.0, 0.1 M) were tried and the enzyme activity was carried out by following the titrimetric method of Birch, Harris and Ray<sup>2</sup>. It was found that 3% sodium chloride was the best extracting solvent for ascorbic acid oxidase of the banana fruit pulp (Table I).

TABLE I  
*Extraction of ascorbic acid oxidase with different solvents*

| Solvent                        | Mg of ascorbic acid oxidised per 100 g wet weight basis in pulp | Ascorbic acid oxidase (units) |
|--------------------------------|---|-------------------------------|
| Water                          | 0.481   | 19                            |
| 30% alcohol                    | 0.672   | 27                            |
| 3% NaCl                        | 1.008   | 40                            |
| Phosphate buffer pH 7.0, 0.1 M | Nil   | Nil                           |

for the determination of ascorbic acid oxidase. Unit activity is defined as the percentage of oxidation of added ascorbic acid and taken to be the criteria of the amount of ascorbic acid oxidase present in tissues and each percentage oxidation represents one unit of the enzyme activity.

It can be seen from Table II that ascorbic acid oxidase activity during storage and ripening at 13° C increased upto yellow stage and then remained steady in the advanced progress of ripening. Fifty per cent ascorbic acid oxidase was observed to be left in an unoxidised form in green unripe stage, whereas in full ripe stage it varied from 35–40%. Babber<sup>3</sup> reported 50% units of ascorbic acid oxidase in bananas. In the present investigation ascorbic acid oxidase ranged in between 38–48% units on fresh weight basis. The highest activity was found in Harichal, Lalkel (48% units) bananas while lowest in Safed velchi banana (45% units). Basrai banana contained 40% units activity and was found to be higher than the other varieties at the initial green stage. There was not much difference in the per cent units activity of Basrai and Rajeli bananas at full stage.

Thanks are due to University Grants Commission for the award of financial assistance to one of us (DLP) for this research. Fresh bananas were supplied by Mr. K. H. Raut is thankfully acknowledged.

TABLE II  
*Enzymatic changes in ascorbic acid oxidase during the ripening of bananas*

| Storage period in days after harvesting |                           | 0<br>(initial stage) | 8     | 16    | 24    | 32       |
|---|---------------------------|----------------------|-------|-------|-------|----------|
| Basrai                                  | Ascorbic acid oxidised mg | 1.01                 | 1.09  | 1.13  | 1.18  | 1.19     |
|   | do. oxidase (units)       | 40.32                | 43.37 | 45.37 | 47.33 | 47.76    |
| Harichal                                | do. oxidised mg           | 1.02                 | 1.10  | 1.15  | 1.19  | 1.20     |
|   | do. oxidase (units)       | 40.32                | 44.28 | 46.30 | 47.76 | 48.23    |
| Lalkel                                  | do. oxidised mg           | 0.87                 | 0.97  | 1.01  | 1.14  | 1.20     |
|   | do. oxidase (units)       | 35.00                | 39.04 | 40.34 | 45.84 | 48.23    |
| Rajeli                                  | do. oxidised mg           | 0.96                 | 1.05  | 1.10  | 1.15  | 1.18     |
|   | do. oxidase (units)       | 38.34                | 42.08 | 44.28 | 46.30 | 47.33    |
| Safed velchi                            | do. oxidised mg           | 0.97                 | 1.03  | 1.09  | 1.13  | Overripe |
|   | do. oxidase (units)       | 39.04                | 41.53 | 43.63 | 45.31 |          |

10 g of banana pulp were homogenised in a Waring blender with 50 ml of 3% sodium chloride for 3–4 minutes. The homogenate was filtered through a double layer of muslin cloth and the residue was again re-extracted with 30 ml of the same solvent to ensure the complete extraction of the enzyme. The combined extracts were kept at 5° C for one hour and then centrifuged at 2,000 r.p.m. for 20 minutes and supernatant layer was made to volume, and used as an enzyme source

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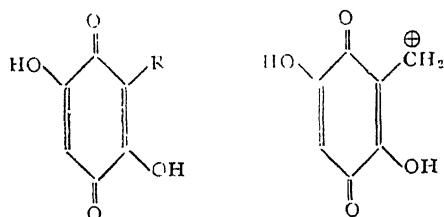
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# MASS-SPECTRAL ANALYSIS OF PIGMENTS FROM *ARDISIA MACROCARPA* WALL

*Ardisia macrocarpa* Wall (Myrsinaceae), a high altitude plant, has been studied for the active principles and isolation of Rapanone (I) and a Leucoanthocyanidin 3, 4, 5, 7, 3', 4', 5'-heptahydroxy flavan has been reported<sup>1</sup>. The identity of the quinone as Rapanone (I) has been established by comparison of I.R., U.V. spectra of the samples of rapanone obtained from *Connorous monocarpus*<sup>2</sup>.

*Connorous monocarpus* yielded a mixture of quinones, whose mass-spectral analysis has been reported<sup>3</sup>. It has been shown to be a mixture of Homo-rapanone (III), Rapanone (I), Embelin (II), and Homo-embelin (IV). This prompted us to study the composition of the pigment obtained from *Ardisia macrocarpa* Wall to clarify whether the composition of the mixture is alike or different from that obtained from *Connorous monocarpus*.

Wood chippings of *Ardisia macrocarpa* Wall, obtained from Nepal, have been extracted and the quinone has been purified by thick-layer chromatography on silica gel using chloroform, methanol (9:1) as eluent. Rapanone which has been suspected to be a mixture has been analyzed mass-spectrometrically, while TLC showed no separation from the authentic sample. The observation of M, (M<sup>+</sup> + 1), and (M<sup>+</sup> + 2) peaks are in agreement with the data reported<sup>4,5</sup>.



- I R = C<sub>13</sub>H<sub>27</sub>  
II R = C<sub>11</sub>H<sub>23</sub>  
III R = C<sub>15</sub>H<sub>31</sub>  
IV R = C<sub>9</sub>H<sub>19</sub>

Rapanone is the major component of the quinone isolated both from *Connorous monocarpus* and *Ardisia macrocarpa* Wall. Mass-spectral data of *Connorous monocarpus* recorded a peak intensity of 90.2 for M<sup>+</sup> of Embelin, while that of pigment from *Ardisia macrocarpa* Wall recorded only 2.3 while that of M of rapanone being 100 in both cases. Mass-spectral data of sample under study indicated the presence of Homo-rapanone (III) (M<sup>+</sup>, 350 7.8%), Rapanone (I) (M<sup>+</sup>, 322 100%), Embelin (II) (M<sup>+</sup>, 294 2.3%) and the side chain fragments are C<sub>14</sub>H<sub>29</sub><sup>+</sup> (197), C<sub>12</sub>H<sub>25</sub><sup>+</sup> (169), C<sub>10</sub>H<sub>21</sub><sup>+</sup> (141). Mass-spectrum showed an intense

peak at M/e 153 (70.2%) which could be attributed to the residue (V).

The pigment isolated from *Ardisia macrocarpa* Wall showed the absence of Homo-embelin (IV), while it is found along with Rapanone (I), Embelin (II) and Homo-rapanone (III) in the sample isolated from *Connorous monocarpus*<sup>3</sup>.

The author is thankful to Professor Dr. Buchard Franck, Director, Organische Chemie Institut, Münster, West-Germany, for the Mass-spectrum.

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## CHEMICAL EXAMINATION OF THE FATS FROM THE SEEDS OF *PHASEOLUS* SPECIES

LEGUMINOSAE is one of the families of plants, seed fats of which contain rarer or higher saturated acids<sup>1</sup>. *Phaseolus trilobus* (Mugani) and *Phaseolus aconitifolius* (Moth) are the species of Leguminosae family belonging to Papilionaceae sub-family. Their seeds come under pulses, and due to high protein content are used as food.

The fats from the seeds of *Phaseolus trilobus* and *Phaseolus aconitifolius* were extracted with 60–80° petroleum ether. The various chemical constants of the fats were determined by standard methods<sup>2</sup>. The physical and chemical constants are tabulated in Table I.

TABLE I  
Physical and chemical constants

| Constants            | <i>Phaseolus trilobus</i> | <i>Phaseolus aconitifolius</i> |
|----------------------|---------------------------|--------------------------------|
| Percentage of fat    | 5.2                       | 4.8                            |
| Specific gravity     | 0.912                     | 0.931                          |
| R.M. value           | 8.2                       | 7.9                            |
| Polenske No.         | 1.5                       | 1.6                            |
| Saponification value | 185                       | 105                            |
| Iodine value         | 44                        | 65                             |
| Thio-cyanogen value  | 57                        | 72                             |
| Acid value           | 27                        | 25                             |
| Acetyl value         | 65                        | 61                             |

I.R.<sup>3</sup>, paper chromatography, T.L.C. methods were used to determine the fatty acid composition of the fats qualitatively by comparing with standard

TABLE II  
Component acids of seed fat of *Phaseolus* species

| Name of species                | Name of bean  | Saturated* |         |           |         |            | Unsaturated* |          |           | Reference |
|--------------------------------|---------------|------------|---------|-----------|---------|------------|--------------|----------|-----------|-----------|
|                                |               | Palmitic   | Stearic | Arachidic | Behenic | Lignoceric | Oleic        | Linoleic | Linolenic |           |
|                                |               | C 16       | C 18    | C 20      | C 22    | C 24       |              |          |           |           |
| <i>Phaseolus lunatus</i>       | Lime bean     | 23.3       | 3.5     | ..        | ..      | ..         | 9.3          | 43.8     | 19.7      | 6         |
| <i>Phaseolus mungo</i>         | Mungo         | 28.0       | 8.0     | 3.0       | ..      | ..         | 18.0         | 40.0     | 3.0       | 7         |
| <i>Phaseolus mungo</i>         | Pulse         | 28.1       | 7.8     | 0.9       | 2.4     | 6.3        | 6.4          | 32.6     | 14.4      | 8         |
| <i>Phaseolus radiatus</i>      | Pulse         | 14.1       | 4.3     | ..        | 9.3     | 3.8        | 20.8         | 16.3     | 35.7      | 8         |
| <i>Phaseolus</i> sp.           | Blackeye bean | 32.5       | 4.6     | ..        | 2.5     | ..         | 7.2          | 31.2     | 22.0      | 6         |
| <i>Phaseolus</i> sp.           | Pinto bean    | 14.7       | 1.0     | ..        | ..      | ..         | 7.0          | 28.1     | 49.2      | 6         |
| <i>Phaseolus vulgaris</i>      | Kidney bean   | 13.4       | 0.7     | ..        | ..      | ..         | 8.3          | 26.9     | 50.6      | 6         |
| <i>Phaseolus trilobus</i>      | Mugani        | ..         | 8.6     | 16.4      | ..      | 21.4       | 51.8         | 1.8      | ..        | This work |
| <i>Phaseolus aconitifolius</i> | Moth          | 25.2       | 4.1     | ..        | ..      | ..         | 10.4         | 39.0     | 21.3      | This work |

\* Percentage by weight.

samples of fatty acids. Quantitative analysis of fatty acids was done by Urea-adduct Fractionation<sup>4,5</sup>. The results are compared with other *Phaseolus* species in Table II.

The major part of the fatty acid composition of the seed fat of *Phaseolus* species is unsaturated fatty acids. It varies from *Phaseolus vulgaris* (86%) to *Phaseolus trilobus* (54%). Linoleic and Linolenic acids are the main component acids of unsaturated acids of the other species but in the case of *Phaseolus trilobus*, Oleic acid (51.8%) is present as the main unsaturated part. Linolenic acid is absent and linoleic acid is in a very small quantity (1.8%) in *Phaseolus trilobus*. In *Phaseolus aconitifolius* all the three unsaturated acids are present in high percentages similar to *Phaseolus radiatus* but the linoleic acid is more than linolenic acid.

Palmitic acid is the main component fatty acid of saturated part in all *Phaseolus* species except in *Phaseolus trilobus*. In this case it is completely absent and large amounts of arachidic acid (16.4%) and lignoceric acid (21.4%) are present. Arachidic and lignoceric acids are in very small quantities in *Phaseolus mungo* and *Phaseolus radiatus*, and also not found in *Phaseolus aconitifolius*. *Phaseolus radiatus* contains 9.3% of behenic acid, but in other species it is absent or present in small quantities. A little higher percentage of stearic acid is observed in *Phaseolus mungo* and *Phaseolus trilobus*.

The small variations in fatty acid composition of the seed fats are possible by variation in ecological and seasonal conditions. But due to lack of data it is not possible to compare the compositions on the basis of ecological and seasonal conditions.

The unsaponifiable matter from the seed fats of *Phaseolus trilobus* (Mugani) and *Phaseolus aconitifolius* (Moth) gave the characteristic sterol reactions and identified as  $\beta$ -sitosterol.

My sincere thanks are due to Dr. R. K. Bajpai and Dr. Alok Singh, for their kind help.

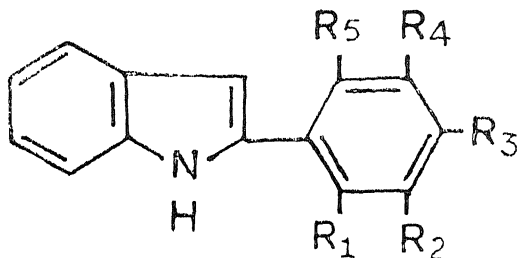
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#### SYNTHESIS OF 2-PHENYLINDOLE DERIVATIVES

IN connection with some synthetic work, we required 2-phenylindoles having alkyl and hydroxyl substituents in that ring. Whilst a few such derivatives are known, not many of them are described in literature. We report here four such new indole derivatives (Table I) conveniently prepared from the

TABLE I



| No. | Indole derivative   | m.p.<br>°C                 | Colour Reaction with |                     |                     | Adducts with sym-tri-nitrobenzene<br>m.p.<br>°C | u.v. and i.r. spectra  |
|-----|---|----------------------------|----------------------|---------------------|---------------------|---|--|
|     |   |                            | HCl/<br>vanillin     | Nitric<br>acid      | Ehrlich<br>reagent  |   |  |
| 1   | R <sub>1</sub> =OH<br>R <sub>2</sub> =CH <sub>3</sub><br>R <sub>3</sub> =CH <sub>3</sub><br>R <sub>4</sub> =R <sub>5</sub> =H | 155<br>(benzene)           | Orange               | Reddish<br>brown    | No colour<br>change | 155<br>(dilute<br>alcohol)                      | $\lambda_{\text{max}}^{\text{MeOH}}$ 225 (log $\epsilon$ 4.321),<br>240 (log $\epsilon$ 4.289),<br>315 (log $\epsilon$ 4.418) n.m., i.r. (KBr)<br>3590 (OH), 3490 (NH of indole),<br>1610 and 1590 cm <sup>-1</sup> (aromatic) |
| 2   | R <sub>3</sub> =OH<br>R <sub>2</sub> =CH <sub>3</sub><br>R <sub>4</sub> =CH <sub>3</sub><br>R <sub>1</sub> =R <sub>5</sub> =H | 248<br>(benzene)           | Blood<br>red         | No colour<br>change | Violet<br>(imm.)    | 164<br>(alcohol)                                | $\lambda_{\text{max}}^{\text{MeOH}}$ 225 (log $\epsilon$ 4.274),<br>245 (log $\epsilon$ 4.422),<br>315 (log $\epsilon$ 4.471) n.m.   |
| 3   | R <sub>1</sub> =OH<br>R <sub>3</sub> =CH <sub>3</sub><br>R <sub>4</sub> =CH <sub>3</sub><br>R <sub>2</sub> =R <sub>5</sub> =H | 162<br>(benzene)           | Blood<br>red         | Orange<br>red       | Violet<br>(imm.)    | 165<br>(dilute<br>alcohol)                      | $\lambda_{\text{max}}^{\text{MeOH}}$ 225 (log $\epsilon$ 4.319),<br>245 (log $\epsilon$ 4.289),<br>325 (log $\epsilon$ 4.406) n.m., i.r. (KBr)<br>3550 (OH), 3400 (NH of indole),<br>1610 and 1590 cm <sup>-1</sup> (aromatic) |
| 4   | R <sub>3</sub> =OH<br>R <sub>2</sub> =CH <sub>3</sub><br>R <sub>5</sub> =CH <sub>3</sub><br>R <sub>1</sub> =R <sub>4</sub> =H | 250<br>(dilute<br>alcohol) | Red                  | No colour<br>change | Violet<br>(imm.)    | ..  | $\lambda_{\text{max}}^{\text{MeOH}}$ 225 (log $\epsilon$ 4.262),<br>245 (log $\epsilon$ 4.258),<br>300 (log $\epsilon$ 4.125) n.m.   |

phenyl-hydrazone of the corresponding hydroxy-dimethyl acetophenones by the application of the Fischer indole synthesis<sup>1</sup> using anhydrous zinc chloride at 170–80°. The yields of the indoles ranged between 15–20%. The colour reactions of these indoles with different reagents, their spectra and their adducts with sym-trinitrobenzene are given. All the four indoles did not give any colour with Ce (SO<sub>4</sub>)<sub>2</sub>. Satisfactory C, H, N analysis for the indoles as well as their adducts were obtained.

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#### ON THE OCCURRENCE OF HALITE IN DIDWANA SALT LAKE AREA, RAJASTHAN

THE objective of this note is to report for the first time, the occurrence of Halite in Didwana salt lake area, situated at a short distance to the south of Didwana town (27° 24' : 74° 34') in the District of Nagour, Rajasthan. The implications of this occurrence on the Indian Geology are: (i) The cause of water salinity (surface and sub-surface), (ii) possibilities of occurrence of rock salt in other salt lake areas and (iii) a definite contribution towards the "Origin of salt in Salt lakes", particularly in Rajasthan.

The Didwana salt lake, producing the salt from subsurface brine for the last 500 years or so, consists of an oval-shaped depression lying in a valley, about 3½ miles in length and about 1½ miles in breadth, running south-west and north-east,

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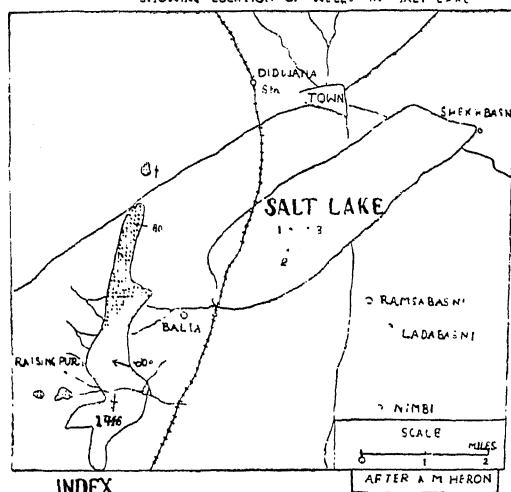


bounded on the western side by an isolated spur mapped as Aravalli's by Heron<sup>1</sup>.

Although the Salt Expert Committee<sup>2</sup> constituted by the Government of India, recommended as far back as 1950, the detailed study of the strata upto the base rock, yet it was only during the last summer that, while digging a well in 'B' Mata Sector (27° 21' 40" : 74° 33' 50") a halite bed was encountered at a depth of 22' 6" from the surface. The halite bed was very hard in digging with the subsurface brine problem and thus could be deepened manually only upto 30' from the surface. The 7½' thick halite occurs here in the form of horizontal beds of variable thickness ranging from 3" to 10" and are intercalated with fine dark grey silt. These beds are overlain by a 5' 10" thick catcarious formation with upper part being lime 'Kankar' deposit which in turn is overlain by a 16' thick dark grey silt deposit, covered by a thin capping of loose sand at the top. In two more wells in the vicinity of the first well, the same formations at about the same depth were noticed. The location of these wells have been shown on the map given below:

#### GEOLOGICAL MAP AROUND DIDWANA

SHOWING LOCATION OF WELLS IN SALT LAKE



#### INDEX

- ☐ ALLUVIUM
- ☐ ARAVALLI QUARTZITE & SLATE

The halite revealed that the halite is pure, greyish white to white in colour, crystalline and waxy. Chemical analysis of one of the samples collected from the first well at depth of 26' from the surface shows a remarkable similarity with the sub-surface brine analysis and the salt analysis as given in Table I.

The similarity in chemical composition clearly indicates that the sub-surface brine in the Didwana salt lake, has resulted from this rock-salt dissolved

TABLE I

| Discovered halite                                  | Sub-surface salt<br>Didwana brine | Didwana salt<br>(4th crop) |
|--|-----------------------------------|----------------------------|
| (Salt Expert Committee Report 1950, pp. 50 and 85) |                                   |                            |
| (Per cent, on dry basis)                           |                                   |                            |
| Sodium chloride                                    | 78.291                            | 83.37                      |
| Sodium sulphate                                    | 16.634                            | 12.39                      |
| Sodium carbonate and bi-carbonate                  | 3.040                             | 4.24                       |
| Undetermined insoluble                             | 2.035                             | ..                         |
| Total  | 100.00                            | 100.00                     |

by subterranean water, which when raised up by the capillary action forms a layer of brine permeating the silt resting on the rock salt. In this context the author would certainly like to mention that the approximate ratio of Sodium Sulphate to Sodium Chloride in the sub-surface brine which is 1 : 3.5 remains same, irrespective of the seasons and fluctuations in sub-surface brine level. Secondly, the wells producing more brine, generally sink down by 5' or so, indicating about the inland sources of the brine in the salt lake, Didwana. Thus the aforesaid discovery contradicts the previously propounded theories of the salt being brought into the lake from outside sources, about the origin of the salt in Rajasthan salt lakes and in particular to the 'wind borne theory' proposed by Christae<sup>3</sup>.

Based on the physiography of the area, bedded nature of the halite deposit and the associated silt and 'Kankar' deposits, it is evident that this halite deposit is younger in contrast to the recently discovered halite occurrence in Bikaner area, in Rajasthan by Sinha, Singh *et al.*<sup>4</sup>. The author tentatively considers this halite as an evaporite deposit of Sub recent to Recent in origin.

The author expresses his thanks to Prof. G. L. Sharma, Head of the Chemistry Department, for his help in chemical analysis of the sample.

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# **BARYTES IN VINDHYAN SEDIMENTS— A NEW FIND**

THE occurrence of barytes was not known so far from the Vindhyan rocks of India. During the course of recent work in the area near Rawat-Bhata (24° 56' : 75° 35'), (Rajasthan), the authors for the first time observed the occurrence of barytes as small veins, stringers, vug fillings and laminations in the Panna shales of upper Vindhyan age in the isolated hillock near Javra Kalan and in the adjoining areas. This new find is interesting both from the point of view of its academic interest and economic significance.

*General Geology* (Vindhyan Super group)

The geological sequence of rocks in the Rawat-Bhata area, between latitudes 24° 55'–25° 00' and longitudes 75° 35'–75° 40' (toposheet No. 45 P/9), is tabulated in Table I.

The barytes in vugs is mostly light pink to whitish in colour with interpenetrating crystals. The crystals are of bigger size in the core and smaller along the margins. The host rocks at the contact of the vugs, also show some disseminations of barytes. Besides barytes, small crystals of pyrite and marcassite are also noticed. A few specks of covellite (?), black tourmaline (?) and fluorite (?) are also present. Such vugs are circular to sub-spherical void in shape and vary in size upto 12 cm.

A prominent vein of about 30 cm width and 8 metres in length is exposed in N 60° E–S 60° W direction in Javra Kalan hillock. A few parallel thin veins and veinlets are also seen trending in the same direction. These veins do not appear to have much depth persistence. As regards the fine laminations of barytes, the occurrence is ubiquitous.

TABLE I

|               | Book succession  | Approx. exposed thickness in metres | Correction based on older nomenclature |
|---------------|--|-------------------------------------|--|
| Bhandar group | Sandstones with interbeds of shales  | 50–70                               | Lower Bhandar sandstones               |
|               | Greenish grey shales   | 25–40                               | Sameria shales (Coulson) <sup>1</sup>  |
|               | Purple and greenish laminated limestones   | 20–30                               | Sirbu shales (Heron)                   |
|               | Brown shales   | 20–30                               | Lower Bhandar limestones               |
| Rewa group    |  | Unconformity                        | Ganurgath shales                       |
|               | Arkosic sandstones   | 1–10                                | Upper Rewa sandstones                  |
|               | Greenish grey shales and siltstones  | 2–30                                | Jhiri shales                           |
|               | Massive sandstones, conglomeratic at the base  | 1–5                                 | Lower Rewa sandstones                  |
|               | Red to purple shales and siltstones with thin laminations, vugs and veins of Barytes | 20–30                               | Panna shales                           |
| Kaimur group  | Gluconitic sandstone, limestone and shales   | 4–10                                |  |
|               |  | Unconformity                        |  |
|               | Red to purple, light grey sandstones with a thin argillaceous band                   | 60–90                               | Kaimur series                          |
| Senri group   |  | Unconformity                        |  |
|               | Greenish grey shales   |                                     | Suket shales                           |
|               |  | Base is not seen                    |  |

Structurally, the rocks of the area are almost horizontal to gently undulating except near the contact beds of Kaimur, Rewa and Bhandar groups which show dips upto 20°–30°, particularly along the western limb of the regional Dipura anticline plunging 3–4° towards northwest.

*Mode of Occurrence of Barytes.*—The barytes observed in the Panna shales occur as vug fillings in the lower horizon of shales and calcareous siltstones (?) near Javra Khurd. The narrow veins and/or joint fillings, laminations and rosettes are noticed in the reddish-purple shales in the upper part of the Panna sequence in the isolated hillock near Javra Kalan.

Chemical analysis of one sample of barytes : BaO 62.71%, SO<sub>4</sub> 32.84%, CaO 0.28%, SrO 0.72%, Mg 600 ppm and Cu 25 ppm, while the host rocks shale contains BaO 8.15%, SO<sub>4</sub> 4.32%, CaO 14.0%, MgO 11.70% and Sr 350 ppm.

*Origin of Barytes.*—The natural abundance of barium in the earth's crust is known to be about 300–500 ppm. Its arrival in the upper crust is thought to be related with the volcanic activity or emplacement of igneous rocks and attended vein systems. In igneous rocks, the basalts contain as little as 100 ppm Ba, granites 700–800 ppm and syenites and potassic igneous rocks have as much as 3000–5000 ppm. Amongst the sedimentary rocks,

particular shales are known to have 500–1000 ppm. Ba<sup>2+3</sup>.

The barytes of sedimentary origin occur either as a continuous bed or lenticular and worm-like bodies or as nodules. The internal texture of the baryte nodules often displays a radiating pattern of crystals from a common centre. The central portion often shows a concentric ring type pattern with the finest material in the centre<sup>4</sup>.

In the light of above background information, and considering the evidences of igneous activity in the Vindhyan basin as a whole, it seems plausible to assume that the origin of the barytes in the present area might be related to some igneous activity as plutonic emplacement or as submarine volcanic emanations in the basin. Since the occurrence of barytes is restricted to the Panna shales horizon, it is obvious to think that the igneous activity, which has brought the barytes in sediments, must have occurred after the deposition of the lower Vindhyan rocks in the basin and such igneous source bodies are concealed under the thick pile of overlying sediments.

**Conclusion.**—The present find of barytes in Vindhyan sediments, besides opening up a new vista in search of economic bodies in the Vindhyan strata, has also afforded clues of igneous activity in the Vindhyan basin of Rajasthan, which was not known so far. It may be mentioned that the occurrence of baryte in Vindhyan rocks of Rawat-Bhata area is entirely a new find and the ideas put forward for its origin by the authors provide scope for locating more such barytes occurrences.

The authors are grateful to Shri S. Narayana-swami, Dy. Director General, G.S.I., Western Region, for his valuable suggestion and editing of the paper.

Geological Survey of India,  
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E. A. KHAN.

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## SEXUAL DIMORPHISM IN THE CAT FISH *MYSTUS (MYSTUS) VITTATUS* (BLOCH)

STERBA<sup>1</sup> describing *Mystus vittatus* states 'Sex distinctions are not known'. Bhatt<sup>2</sup>, however, working on the biology of the fish, mentions about sexual dimorphism—'Sexual dimorphism in *Mystus vittatus* is of permanent nature and can be easily seen in the males in the form of a genital papilla. This papilla is a projection of the genital aperture and varies from 2 mm to almost 10 mm in length. The genital papilla is seen throughout the year and gets more enlarged during the spawning season.'

For the present study more than one thousand specimens were examined in the months of May and June. The fishes were collected from the local lakes and rivers. Their size ranged between 70 mm–120 mm for the males and 70 mm–145 mm for females. The male and the female ratio was found to be approximately 1:1.3.

While the males possess a well-developed genital papilla as reported by Bhatt<sup>2</sup> (Fig. 1) they also display in addition a spear-shaped thickening at the base of the caudal fin near the level of the lateral line and almost extending upto the fork (Fig. 1).

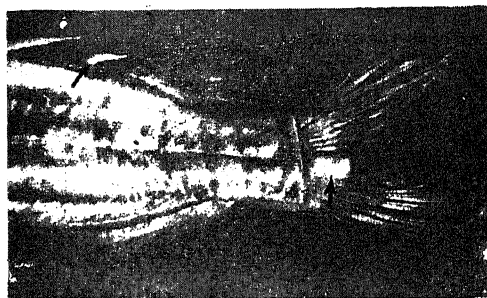


FIG. 1. Photograph of the posterior half of an adult *Mystus vittatus* showing the genital papilla and the thickening between 7th and 9th fin rays (arrows),  $\times 1.7$ .

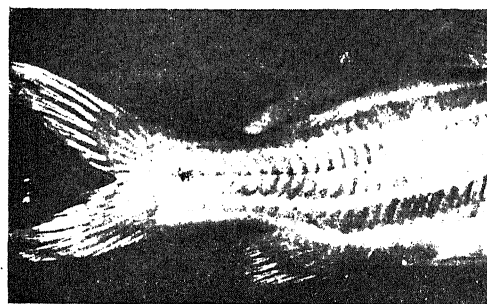


FIG. 2. Photograph of the posterior half of a female *M. vittatus*,  $\times 1.5$ .

It lies between the seventh and the ninth fin rays embracing their basal part and more or less envelop-

ing the eighth fin ray. Males, measuring below 80 mm approximately do not show this thickening hence it may be taken as a secondary sexual character. The females do not possess any of the characters mentioned above for the males (Fig. 2). Thus, it is very easy to identify the sex of this species at the first sight. And also, the thickening stands as an indicator to know the maturity of the male fish.

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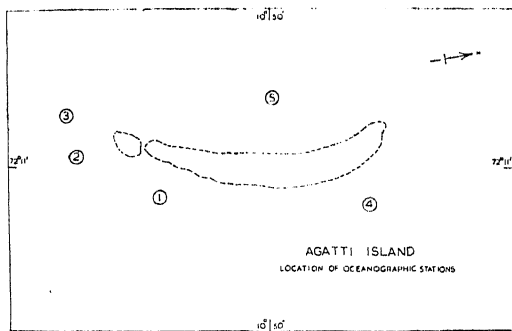
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#### A NOTE ON TUNA FISHERY AROUND AGATTI ISLAND (LAKSHADWEEP) IN RELATION TO HYDROGRAPHIC CONDITIONS LEADING TO THE PHENOMENON OF UPWELLING

HYDROGRAPHIC studies conducted by Rao and Jayaraman (1966) around Minicoy islands indicated the presence of upwelling during late November and suggested that this phenomenon may have considerable impact on the peak tuna catches in this region.

The present observations are based on meteorological, hydrographic and fishing data collected by the training-cum-fishing vessels 'Blue Fin' and 'Masterfisherman I' attached to the Central Institute of Fisheries Operatives. Both the vessels were on a 10 day cruise to Lakshadweep islands during the 2nd week of December (1973).

Being the only island in the group from where moderate to good fishing for skipjack tuna (*Katsuwonus pelamis*) was reported during the season, both meteorological and hydrographic data were collected from 5 fixed oceanographic stations around the Agatti island, side by side with pole and line fishing operations.



Large shoals of skipjack tuna were observed especially on the southern and south-western side of the island. The fishes caught by the pole and line fishing boats were more or less of the same size group (approximately 18" in length) and weighed on an average between 2 kg and 2.5 kg. The fishing was moderately good and on an average each pole and line fishing vessel could catch a minimum of around 100 numbers of tuna within a period of one hour. The catch per hour for the above-mentioned individual species of tuna worked out to approximately 250 kg especially of those boats which were fishing on the southern and south-western side of the island.

The prevailing surface currents were north-westerly to north-north-westerly and the velocity of the flow was moderately high. The surface water temperature varied between 28.0° C and 28.1° C and on an average the isothermal layer was found to extend to depths of 50 mtrs. to 55 mtrs. from where the thermocline was found to extend to depths of 90-95 mtrs. at more or less all the stations. The surface salinity varied between 34.799‰ and 35.028‰ with the maximum concentration of the shoals at regions of comparatively higher salinities.

It was observed that the surface currents which head towards the island on its southern tip diverge into two branches one on the eastern side and the other on the western side. The comparatively higher salinity values and low temperature values observed at the surface levels on the southern side of the island indicate the possibility of upwelled water in this area brought from sub-surface levels through the process of upwelling.

It is known that tuna gather around areas of upwelling and in areas where the thermocline is shallower (Nakagome, 1973). Studies conducted in the Caroline and Marshall islands have revealed that better catches of Big eye tuna was brought in during the season when the sea surface temperature was comparatively lower (Nakagome, 1958, 1965). Uda and Nakamura (1973) have observed that the region of maximum hook rate appears to be localised either in the marginal area, water boundaries or along oceanic fronts.

Thus the concentration of large shoals of tuna on the southern side of the island may perhaps indicate a possible relationship between the occurrence of tuna shoals and upwelling zones. It is worth mentioning in this context that in the year 1966 in the presence of diverging currents and the resulting upwelling a good fishery for skipjack tuna flourished around Minicoy island. It seems quite likely that the divergence zone which leads to a favourable environment is shifting from one area to another possibly depending on the velocity and

direction of the prevailing current system, geographical location of the islands, bottom topography of the atolls, etc. It may be possible that probable fishing grounds for skipjack tuna in the Lakshadweep area of the Arabian Sea could be predicted sufficiently in advance, by keeping a constant watch on the formation and shifting of divergence zones around the islands during the period September to April, the season for the skipjack tuna fishery. Further investigations in this line are worth taking up.

The authors wish to express their sincere thanks to Dr. A. V. S. Murthy, Fishery Scientist (Oceanography), Central Marine Fisheries Research Institute (ICAR), for the helpful discussions, Shri George Varghese, Director of Fisheries, Lakshadweep, for making available the facilities in the form of tuna pole and line fishing vessels and gear and to the skippers of the training vessels, Capt. K. Balan and Capt. R. Velayudhan and the crew for their sincere cooperation.

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#### A NOTE ON THE CHANGES IN SOME QUALITATIVE CHARACTERS DURING THE LAST PHASE OF MATURATION OF INDIAN MUSTARD SEED (*BRASSICA JUNCEA*, COSS)

DURING the later stages of maturation of an oilseed plant several changes occur in the seed very rapidly. The seeds shed lot of moisture, accumulate more dry matter, rate of oil formation increases rapidly and other changes occur in the composition of oil. Simultaneous with oil formation the fat content (dry wt. basis) and fat out-turn per unit number of seeds first increases upto a point and then slightly comes down at the end of dead ripening stage<sup>1</sup>.

The other important constituents in the seeds are the fatty acids of the oil. Erucic acid (major acid in rapeseeds and mustards) did not usually become the major acid until 35 days after flowering in *Sinapis alba*<sup>2</sup>. In the earlier stages saturated acids

were high in percentage but came down gradually as the seeds matured. In the end erucic acid accounted for nearly 45% of the total acids.

A study was made with a variety of commonly cultivated Indian mustard (*B. juncea* Coss) var. Appressed Mutant to observe these changes during maturation.

**Experimental.**—Sample of seeds were collected from a crop of mustard var. Appressed Mutant starting from 75 days after sowing and at an interval of four days till 95th day. The pods were shattering type and, therefore, were harvested when just turned yellow. Moisture percentage was determined by drying the seeds in an oven at 120°C for 2 hours. Oil was extracted in Soxhlet's apparatus for 8 hours with petroleum ether B.P. 60–80°C., after crushing the seeds thoroughly in a mortar. Fatty acids were analysed in Gas Liquid Chromatograph. Methyl esters were prepared for this purpose by transmethylation with 14% BF<sub>3</sub>-Methanol<sup>3</sup>. The esters were analysed in a F and M model 700–12 analytical g.l.c., with a dual flame ionization detector and a 6' × 1/8" stainless steel column packed with 10% D.E.G.S. supported on Gas Chrome P (60–80, mesh). Operating conditions were oven temp. 180°C, chart speed 15 inch/hour and range 10<sup>4</sup>. The carrier gas was nitrogen. The area under a 'peak' was measured by the method of retention time × peak height<sup>4</sup> and the percentages were calculated by 'Area Normalization method'<sup>5</sup>. All the estimations were done with duplicate samples.

TABLE I  
Changes in chemical composition during maturation of mustard seeds

| Days after sowing | Moisture % | Oil % | Dry wt. of 100 seeds in mg | Oil out-turn/ 100 seeds (dry wt. basis in mg) |
|-------------------|------------|-------|----------------------------|---|
| 75                | 58.03      | 19.60 | 117.2                      | 54.73   |
| 79                | 57.78      | 20.39 | 136.0                      | 65.68   |
| 83                | 56.08      | 22.23 | 160.1                      | 81.03   |
| 87                | 52.67      | 23.04 | 169.7                      | 82.61   |
| 91                | 50.55      | 24.67 | 180.5                      | 90.05   |
| 95                | 32.35      | 27.18 | 208.2                      | 83.61   |

**Results and Discussion.**—Some of the quality factors have been presented in Table I. The moisture percentage came down steadily, but at the last stage the decrease was very rapid resulting in a lowering of 18%. The oil percentage on fresh weight basis increased steadily and it appears that there was a quicker synthesis of oil during the last week under study. Similar trend was observed in the case of seed weight. There was a 100% increase in weight in about 20 days. Weight of oil per unit number of seed increased till 91st day, then there was a sud-

den drop of 7 mg. A similar trend<sup>1</sup> was also observed in oil quantity during the last stage of maturation elsewhere. It is obvious from the data that the synthesis of oil and increase in dry matter both goes on at a faster rate during the last phase of maturity. At the same time there is rapid depletion of moisture from the seed. In this particular variety the moisture comes down to 12% after drying and threshing the crop, which incidentally increases the oil percentage to nearly 33–35%. This of course does not mean that any oil synthesis takes place in this period. It will be also obvious from the last column that actual percentage of oil (on dry wt. basis) comes down during the last four days, implying thereby, that synthesis either stops or slows down very considerably.

One of the quality aspects of the oil is the fatty acid composition. This is becoming an important consideration as more and more people are becoming conscious of it and also clinical studies are being undertaken to evaluate any harmful effect of the oil on human beings. In this study (Table II)

TABLE II

Fatty acid percentages by weight of eight major fatty acids in maturing mustard seeds

| Fatty acid | Days after sowing |       |       |       |       |       |
|------------|-------------------|-------|-------|-------|-------|-------|
|            | 75                | 79    | 83    | 87    | 91    | 95    |
| C 16 : 0   | 3.15              | 3.98  | 3.83  | 3.54  | 3.23  | 2.09  |
| C 18 : 0   | 2.41              | 2.54  | 1.87  | 1.89  | 1.46  | 1.13  |
| C 18 : 1   | 8.90              | 9.34  | 10.24 | 8.24  | 9.58  | 9.12  |
| C 18 : 2   | 15.80             | 15.92 | 15.50 | 13.98 | 14.96 | 13.71 |
| C 18 : 3   | 12.80             | 12.16 | 16.36 | 15.86 | 11.89 | 12.71 |
| C 20 : 1   | 6.18              | 6.04  | 8.40  | 7.93  | 6.53  | 5.40  |
| C 20 : 2   | 1.30              | 1.35  | 1.55  | 0.72  | 1.32  | 0.94  |
| C 22 : 1   | 49.46             | 48.69 | 42.25 | 47.84 | 51.03 | 54.10 |

the percentages of saturated fatty acids and C 18 : 2 (linoleic) came down gradually during maturation while among the unsaturates, C 18 : 1, C 18 : 3, C 20 : 1, and C 20 : 2 increased upto 83 days; then there was a gradual decrease. The erucic acid (C 22 : 1) showed an erratic trend. It increased from 87th day till 95th day. Before this period there was a gradual decrease. Similar type of observation has been made by others<sup>2</sup>. In the light of present theories of fatty acid biosynthesis, there is simultaneous desaturation from Oleic to higher homologues and a chain elongation process from Oleic to Eicosenoic and finally to Erucic<sup>3</sup>. The latter process seems to be more in evidence in this study, the desaturation process showing little impact on the synthesis pattern of the fatty acids. There could be of course a possibility that the chain elongation was a faster process thereby giving less

scope to the precursor to be desaturated at a very slow process to its higher homologues. These studies may help in understanding the quick changes that occur during maturation of an oilseed, specially in determining the optimum harvest stage from the quality point of view.

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## CURRENT POSITION OF PHYSIOLOGIC RACES OF *PYRICULARIA ORYZAE* CAV. IN INDIA

THE first report on the occurrence of physiologic races in the fungal pathogen, *Pyricularia oryzae* Cav. in India was by Padmanabhan<sup>1</sup>. Padmanabhan *et al.*<sup>2</sup> reported the occurrence of thirty-one races of the pathogen. A report<sup>3</sup> from Central Rice Research Institute added one more race of the pathogen to the list.

The current contribution covers the present position of prevalence of races of *P. oryzae* in India for the period 1972 and 1973.

The isolates of the pathogen utilized in the study were obtained by isolation from blast specimens collected from Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Orissa, West Bengal, Assam, Bihar, Madhya Pradesh, Uttar Pradesh, Himachal Pradesh, Jammu and Kashmir, Rajasthan, Gujarat and Maharashtra. One hundred and eight isolates of *P. oryzae* were isolated and identified.

The international set of eight differential rice varieties, viz., Ramínad str. 3, Zenith, NP. 125, Usen, Duilar, Kanto-51, CI 8970(S) and Caloro were utilised for the experiment. The susceptible rice variety, Co. 13, was utilised as a check since the occurrence of an isolate of the race II (non-infective on the eight differentials but pathogenic on Co. 13) has been reported by Padmanabhan *et al.*<sup>2</sup>.

The method adopted for identification of races of the pathogen was the one described by Padmanabhan

*et al.*<sup>4</sup>. The classification of races of *P. oryzae* of Ling and Ou<sup>5</sup> was adopted.

The results are presented in Table I.

TABLE I  
Composition and distribution of races of *P. oryzae*

| Race  | Year |      |
|-------|------|------|
|       | 1972 | 1973 |
| IC-1  | 2    | Nil  |
| IC-17 | 46   | 60   |

The race IC-1 could be identified from the isolates belonging to Orissa and Rajasthan. The race IC-17 could be identified from amongst the isolates from all the states mentioned above. The variety Co. 13 proved susceptible to all the one hundred and eight isolates of *P. oryzae*.

The situation reveals that the isolates belonging to the IC group are more predominant and more stable than all other races. Padmanabhan *et al.*<sup>2</sup> reported that the most prevalent races in India were IC-3 followed by ID-1 (Atkins *et al.* classification<sup>6</sup>), i.e., IC-17, ID-1 (Ling and Ou classification)<sup>5</sup>. The reactions of the differential varieties to race IC-1 and IC-17 are presented in Table II.

TABLE II  
Reaction of differential varieties to race IC-1 and IC-17 of *P. oryzae*

| Variety | Raminad<br>str. 3 | Zenith | N.P. 125 | Usen | Dular | Kanto-51 | CI. 8970(S) | Caloro |
|---------|-------------------|--------|----------|------|-------|----------|-------------|--------|
| Race    |                   |        |          |      |       |          |             |        |
| IC-1    | R                 | R      | S        | S    | S     | S        | S           | S      |
| IC-17   | R                 | R      | S        | R    | S     | S        | S           | S      |

The difference between the reaction of the test isolates of the variety Usen, i.e., whether the infection manifested itself as a circular spot with central ashy zone and dark purplish brown margin classified as 'C' spot, or as a broadly spindle-shaped spot 'D' as described by Padmanabhan *et al.*<sup>4</sup> decides whether the variety behaved as resistant or susceptible.

Both 'C' and 'D' spots reveal central ashy zone, brown margin and sporulation. Besides, there are lesions which could be falling between 'C' and 'D' types of spots. Therefore it is proposed that the 'C' type spots may be classified as indicative of a susceptible reaction. Sometimes isolates of *P. oryzae* occurring in India produce a border-line reaction on the variety, Usen, which lies between resistance and susceptibility (C and D type spots). It is therefore necessary to replace the variety, 'Usen' with a short duration variety with a clear-cut reaction like, Co. 13.

Help received from scientists belonging to the Department of Agriculture of the different States of India and Universities in providing blast specimens is gratefully acknowledged. Help rendered by Sri N. K. Chyau Patnaik, Assistant Botanist, in maintaining the purity of the differentials and supply of seeds is also gratefully acknowledged. The authors are grateful to Dr. S. Y. Padmanabhan for his keen interest and encouragement.

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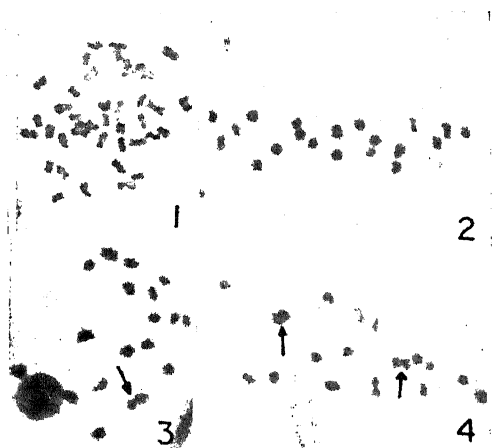
## CYTOLOGY OF *PASPALUM SCROBICULATUM* LINN.

THE genus *Paspalum* Linn. consists of 350 species widely distributed in the tropics and subtropics of both the hemispheres<sup>1</sup>. In India this genus is represented by 14 species<sup>2</sup>. *Paspalum scrobiculatum* Linn. is an important minor millet chiefly cultivated in India. The grain is used for preparing pudding or porridge. The chromosome number has been reported to be  $2n = 40$  and  $n = 20$  in this species<sup>3,4</sup>. The karyotype and meiotic behaviour, however, have not been investigated. This communication deals with these aspects.

The seeds were obtained through the courtesy of Botanical Survey of India, Southern Circle, Coimbatore. For karyotype study the root tips were excised from the potted plants and pretreated with 0.002 molar 8-hydroxyquinoline for 3 hours. They were stained with Lillie's (1951)<sup>5</sup> Schiff's reagent. Spikes were fixed in Carnoy's fluid (6:3:1) and microsporocytes were stained with acetocarmine. The slides were made permanent using acetic acid-butanol series and mounted in euparal.

The present study confirms the earlier counts of  $2n = 40$  and  $n = 20$  chromosomes. The somatic chromosomes are shown in Fig. 1. They are medium sized. The difference between longest and shortest chromosome in the complement is very

small. Therefore they cannot be categorized into long, medium and short chromosomes. They form a gradual series. There are three types of chromosomes, a single satellited submedian pair with SAT on long arm, eight pairs of median chromosomes and eleven pairs of chromosomes with submedian centromere. The satellite is small and measures about 0.5 micron. The chromosome length ranges from 1.9 to 3.5 microns with an absolute length of 103.9 microns.



Figs. 1-4. Fig. 1. Somatic metaphase plate showing  $2n = 40$  chromosomes. Fig. 2. Metaphase I with 20 bivalents. Fig. 3. Diakinesis showing 18 bivalents and 1 quadrivalent ( $\uparrow$ ). Fig. 4. Metaphase I with 16 bivalents and 2 quadrivalents ( $\uparrow$ ). All Figs.  $\times 900$ .

The meiotic behaviour is studied in detail. Twenty bivalents are observed at diakinesis and metaphase I (Fig. 2). The bivalents are mostly ring shaped. However, approximately 10% of the PMC's contain 1-2 quadrivalents (Figs. 3-4). Anaphasic separation at first and second division is mostly normal. Sporadically 1-2 lagging chromosomes are found at anaphase I and II. The tetrad formation is regular.

From the available evidences<sup>6</sup> it appears that 10 is the basic chromosome number in *Paspalum*. Thus *P. scrobiculatum* with  $2n = 40$  chromosomes is a tetraploid. Regular 20 bivalent formation at meiosis indicates its allotetraploid nature. Occurrence of small percentage of quadrivalents possibly suggests that this species is a segmental allotetraploid.

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### INDUCED FLORAL MUTATION IN *PHYSALIS IXOCARPA* BROT.

NUMEROUS studies in recent years have shown that induced mutations can offer a large number of morphological variations in different parts of the plant<sup>1-4</sup>. So far there is no report on the mutagenesis in *Physalis*. Modifications in the flower and fruit of *P. ixocarpa* mutant and genetical studies of the same have been reported here.

Seeds collected from a single plant formed the stock of present study, were treated with chemical [dimethyl sulphate (DMS), diethyl sulphate (DES) and methyl ethane sulphonate (MES)] and physical (gamma rays) mutagens. The various mutagens were used to study their comparative efficiency in *Physalis*. Dry seeds pre-soaked in water for 12 hours were used for chemical treatments (0.25, 0.50, 0.75 and 1.0% by volume). They were irradiated as such with 20-40 kR of gamma rays. The pre-soaked seeds were treated with freshly prepared aqueous solution of chemicals for 12 hours. The treatment was carried out in test tubes which were intermittently shaken. The seeds treated with chemicals were washed thoroughly with tap water and sown in the nursery. The resulting seedlings were then transplanted in the field to obtain  $M_1$  population. In all four mutant plants were isolated from 0.50 and 1.0% DES, 0.75% DMS and 20 kR treatments respectively, but no mutant plant could be isolated from MES treatments.

The conspicuous floral changes in the mutant were bud open from very young stage (Fig. 1 E), style projecting in bud (Fig. 1 F) and corolla showed slow rate of growth with a very short tube. Stamens which in normal flowers occur in the centre and surrounded the ovary closely (Fig. 1 C; Fig. 2 A) were placed wide apart with exposed ovary projecting above all the floral parts (Fig. 1 G; Fig. 2 D, E). After pollination the fruit developed rapidly and



since the ovary was protruding, the enlarged calyx failed to enclose it. The latter occurred as a cap-like structure at the base of fruit giving curious

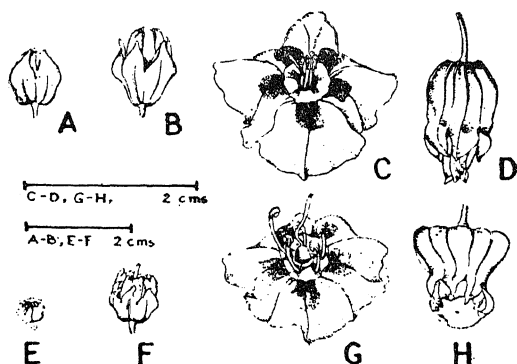


FIG. 1

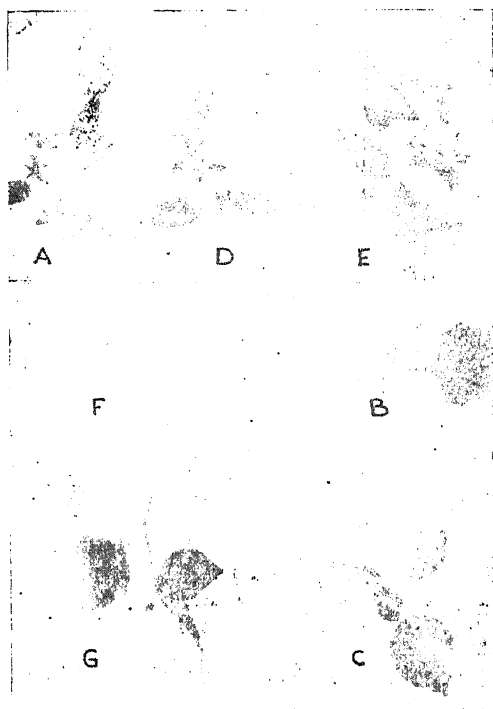


FIG. 2

FIGS. 1-2. Fig. 1 A-H. (Diagrammatic), Comparison of floral characters of control and mutant of *P. ixocarpa*. A-D, Control, note the closed bud in A and aggregated stamens in C. E-H, Mutant showing open bud (E, F) and separated stamens in G. Fig. 2 A-G (Photographs). Flower and fruit characters of control and mutant of *P. ixocarpa*. A-C, Control note aggregated stamens in A and fruit enclosed in enlarged calyx (B, C). D-G, Mutant showing separated stamens in D, E and cap-like structure at the base of fruit (F, G).

look to the mutant (Fig. 1 H; Fig. 2 F, G) as compared to the control in which the fruit was completely enclosed by the enlarged calyx (Fig. 1 D; Fig. 2 B, C).

The mutant on isolation showed poor fruit setting, with thin membranous seeds. All efforts to induce seeds to germinate in petri dishes, pots, paper towels and germination chamber failed. A study of style and stigma after 48 hours of pollination showed that pollen grains germinated and the pollen tube travelled down the style. The mutant also showed 98% pollen fertility.

The seeds obtained from the cross of mutant and control plant germinated readily and progeny segregated in almost 1:1 ratio for open bud *versus* normal (closed) bud (Fig. 1 A, B) character. The open bud individuals in  $F_1$  behaved like the parent and in each back cross again gave 1:1 ratio.

These observations on the mutant behaviour in self and cross-pollination revealed that the original mutant was heterozygous for open bud factor which showed marked pleiotropic effects on other floral and fruit characters. The mutation was a dominant one and since homozygous for gene could not be recovered, the gene in homozygous form might be lethal to the mutant.

As the mutant plant in heterozygous form is viable, the drastic effects in floral characters make it a very useful type for breeding project. The open bud condition, projecting style, widely placed stamens and raised ovary provide the mechanism to ensure cross-pollination in the mutant. The open bud condition gives it the major advantage to carry out emasculation with ease and least injury.

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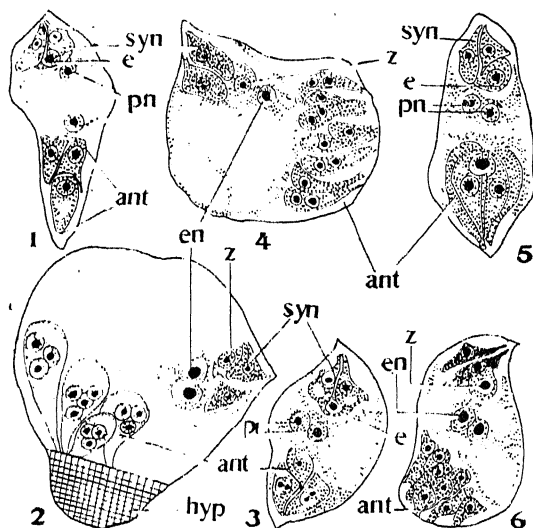
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#### ANTIPODAL CELLS IN SOME MEMBERS OF GRAMINEAE

REPORTS on the ephemeral, as well as persistent, large sized, supernumerary and multinucleate antipodals are available<sup>1</sup>. Gramineae are no exception and secondary multiplication of antipodals is the characteristic of this family<sup>3</sup>. *Eleusine indica*, *E. coracana* and *Bromus inermis* show a considerable increase in size of the antipodal cells<sup>2,5,7</sup>. As many as three hundred antipodal cells have been recorded in *Sasa paniculata*<sup>3</sup>, while in *Setaria*

*italica*<sup>6</sup> they are multinucleate. This paper deals with the study of the antipodal cells in *Eleusine africana* Kennedy-O Byrne, *E. compressa* (Forssk.) Aschers. et Schweing and *Chloris gayana* Kunth, members of the Gramineae.

In *Eleusine africana*, *E. compressa* and *Chloris gayana* the development of the embryo sac is of the Polygonum type, and it is conical in shape. In *E. africana* (Fig. 1) there is a deep narrow chalazal pouch. The egg apparatus is typical with two pyriform laterally beaked and apically hooked synergids. The filiform apparatus is present. In *E. africana* the two polar nuclei are separated by a central vacuole while in *E. compressa* and *C. gayana* they are in the centre. In all the three species the antipodal cells before the fusion of polar nuclei are three and they are large and uninucleate (Figs. 1, 3, 5). In *C. gayana* (Fig. 5) they are very large and occupy more than half the space of the embryo sac. Further, in *C. gayana* they are prominently beaked, the beaks being pointed towards the chalazal end. Except in one of the cells of the antipodals in *E. africana* in the rest the vacuoles are not present. On the other hand, they are filled completely with cytoplasm and stain deeply.



FIGS. 1-6. Figs. 1-2. Embryo sac in *Eleusine africana*; (ant; antipodal cell; e, egg; en, endosperm nuclei; pn, polar nuclei; sn, secondary nuclei; syn, synergids; z, zygote; hyp, hypostase). Note three uninucleate antipodals in Fig. 1 and four multinucleate antipodal cells in Fig. 2.  $\times 200$ . Figs. 3-4. *E. compressa*; same as in Figs. 1 and 2 respectively but in Fig. 4 antipodal cells are many.  $\times 125$ . Figs. 5-6. *Chloris gayana*; Embryo sac before fertilisation (Fig. 5) and after fertilisation (Fig. 6); note many antipodal cells and two endosperm nuclei,  $\times 200$ .

After the fusion of the polar nuclei, the embryo sacs of *E. africana*, *E. compressa* and *C. gayana* enlarge and almost become spherical (Figs. 2, 4, 6). In *E. africana* (Fig. 2) and *E. compressa* (Fig. 4) the antipodal cells are four and eight respectively due to the division of one or more cells. However, they are three or four nucleate in *E. africana* and one or two nucleate in *E. compressa*. In both the species they occupy the lateral position of the embryo sac and are baloon-shaped. In *C. gayana* (Fig. 6) the large antipodal cells have divided to form five to eight narrow baloon-like cells which are uninucleate and placed at the chalazal end. Fusion of the nuclei was not observed in any of these antipodal cells. In *E. africana*, a hypostase is formed at the chalazal end just below the antipodal cells (Fig. 2).

In *E. coracana*, *E. indica* and *Dactyloctenium aegypticum* the antipodal cells are quite large and uninucleate<sup>5, 7</sup>. In *E. coracana* and *E. indica* the antipodal cells neither divide nor become coenocytic. Rarely in *E. coracana* division may take place. But in *D. aegypticum* the antipodal cells divide further to form six to ten cells and become coenocytic. In *Pennisetum typhoideum* three to eight nuclei have been recorded in each antipodal cell which finally fuse<sup>1</sup>.

Various functions have been attributed to antipodal cells<sup>5</sup>. Looking into their size, indefinite number, multinucleate condition and high staining capacity they may take part in the nutrition of the embryo sac. Further investigations on the above species and also on *Eragrostis superba*, *Cynodon dactylon* and *Paspalum commersoni* are in progress.

The award of Junior Research Fellowship by the University Grants Commission, New Delhi, to one of us (M. S. M.) is gratefully acknowledged.

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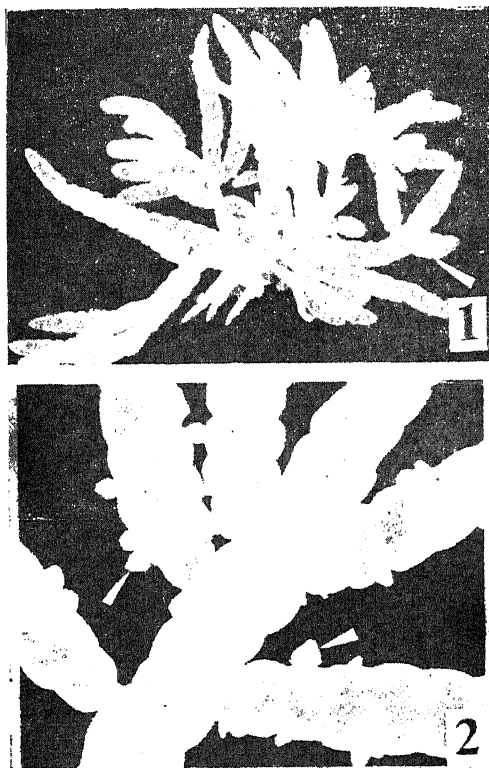
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ON *GASTROCLONIUM IYENGARII* SRINIVASAN

*Gastroclonium iyengarii* Srinivasan<sup>1</sup> was based on tetrasporic specimens collected first by Srinivasan himself and later by Desikachary from Okla. In some collections made at Porbandar and Veraval, the writer came across a few cystocarpic specimens of this species and a critical study of these was made. Some of the observations are very briefly presented here.

The cystocarpic plants were rather small, though conforming to the typical morphology of this species as described by Srinivasan (Fig. 1). However, one notable difference from the type seen in the present material was the presence of well-developed secondary ramuli. This is particularly well seen in Fig. 1 (lower left, and to a slightly lesser extent, lower right). Srinivasan<sup>1</sup> has stated that the "secondary ramuli are generally absent, but occasionally present, and, when present, rudimentary".



FIGS. 1-2. Fig. 1. Tetrasporic plant showing general habit and well-developed secondary ramuli (arrow),  $\times 1.50$ . Fig. 2. Portion of cystocarpic plant enlarged to show clearly ostiolate cystocarps (arrows),  $\times 4.50$ .

The cystocarps are  $800-1000\mu$  in diameter and approximately of the same height, urceolate, and

with well-defined ostioles (Fig. 2, arrows). It was a surprise to note that the cystocarps were ostiolate. For, in all previous accounts the cystocarp of *Gastroclonium* is described as 'non-ostiolate'. In fact, of the 4 genera included by Kylin<sup>2</sup> in his "*Champia* Gruppe" (= Subfamily Champieae) only *Champia* is considered to have ostiolate cystocarps and this feature has been used by Kylin (*l.c.*, p. 345) to key out *Champia* from the three other genera, viz., *Chylocladia*, *Coeloseira* and *Gastroclonium*. The presence of ostiolate cystocarps in *G. iyengarii* would seem to invalidate this distinction.

Thanks are due to Shri N. R. Mankad for material collected at Porbandar.

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## MEIOTIC STUDIES IN SPECIES AND HYBRIDS IN MEDICINAL YAMS

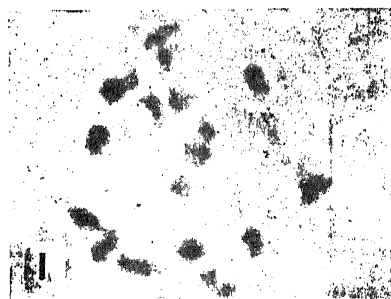
SPECIES of the genus *Dioscorea* L. are cultivated for their edible tubers popularly known as the yams and for the extraction of diosgenin, a source material for the synthesis of steroid hormones. In a breeding programme aimed at the improvement of the medicinal yams, three Mexican species of *Dioscorea*, viz., *D. floribunda* Mart and Gal., *D. composita* Hemsl. and *D. friedrichsthallii* Kunth have been crossed and hybrids obtained<sup>1</sup>. The present note gives an account of the cytology of the species and hybrids.

Meiosis was studied in iron acetocarmine smears of the PMC's. The chromosomes were very small ranging from  $0.3\mu$  to  $1.5\mu$  at MI. Meiosis was regular (Figs. 1 and 2) in the three species with the regular formation of 18 bivalents. The half chiasma frequencies per chromosome were 1.64, 1.61 and 1.52 in *D. floribunda*, *D. composita* and *D. friedrichsthallii* respectively (Table 1). With the present technique employed, it was not possible to find out the presence or otherwise of any sex chromosomes. Since the basic chromosome number of the new *Dioscorea* species is 92, and since meiosis is regular in the 3 species, they may probably represent allotetraploids ( $2n=36$ ) containing 2 genomes, the homologies of which are not known.

The hybrids had fairly regular meiosis except for the presence of a few univalents and higher associations involving 3 and 4 chromosomes (Table I). It could not be made out whether these chromosome associations represent multivalents or

TABLE I  
Frequency of chromosome associations in species and hybrids of *Dioscorea*

| Sl. No. | Species   | No. of cells analysed | Frequency of chromosome associations |      |       |      | Mean No. of $\frac{1}{2}$ chiasmata per chromosome |
|---------|---|-----------------------|--------------------------------------|------|-------|------|--|
| 1.      | <i>D. floribunda</i>                                      | 50                    | ..                                   | 18   | ..    | ..   | 1.64   |
| 2.      | <i>D. composita</i>                                       | .. 50                 | ..                                   | 18   | ..    | ..   | 1.61   |
| 3.      | <i>D. friedrichsthallii</i>                               | .. 50                 | ..                                   | 18   | ..    | ..   | 1.52   |
| 4.      | <i>D. composita</i> $\times$ <i>D. floribunda</i>         | .. 18                 | Total mean                           | 13   | 293   | 3    | 10   |
| 5.      | <i>D. floribunda</i> $\times$ <i>D. composita</i>         | .. 10                 | ..                                   | 0.72 | 16.7  | 0.17 | 0.56   |
| 6.      | <i>D. floribunda</i> $\times$ <i>D. friedrichsthallii</i> | 21                    | ..                                   | 3    | 171   | 1    | 3  |
| 7.      | <i>D. friedrichsthallii</i> $\times$ <i>D. floribunda</i> | 21                    | ..                                   | 0.30 | 17.10 | 0.1  | 3.3  |
|         |   |                       |                                      | 5    | 348   | 5    | 10   |
|         |   |                       |                                      | 0.24 | 16.57 | 0.24 | 0.49   |
|         |   |                       |                                      | 14   | 362   | 2    | 3  |
|         |   |                       |                                      | 0.67 | 17.23 | 0.09 | 0.14   |



FIGS. 1-3. Figs. 1-2. Diakinesis in *D. floribunda* (18 II). Fig. 3. Diakinesis in the hybrid *D. floribunda*  $\times$  *D. composita*. Showing 14 II and 2 IV (arrows).

those formed due to reciprocal translocations (Fig. 3). Meiotic behaviour of the species alone, however, does not rule out completely the former possibility since such a diploid behaviour of the species could result from a genetic control of chromosome pairing or a gradual diploidisation of homo- or homeologous genomes. When such genomes are brought together in a new combination, it is quite possible for the original homologies to get expressed due to an altered genetic background. The male and female fertilities of the hybrids lend less support to the existence of reciprocal structural changes.

I.I.H.R., Bangalore-6,  
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# OCCURRENCE OF GALLMIDGE, *ASPHONDYLIA* SP. (CECIDOMYIIDAE: DIPTERA) AS A PEST OF BRINJAL (*SOLANUM MELONGENA* LINN.) IN INDIA

BRINJAL (egg plant) fruits were found infested with the gall midge, *Asphondylia* sp. at the experimental farm of the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, for the first time in spring 1973. Maggots feed from inside on the ovary of the flower buds and flowers and on the tissues of the developing fruits. When young flowers are attacked the stamens and pistil degenerate and fruits fail to form. Damage by maggot during development of the fruit is confined to the part of the fruit that is being attacked but nevertheless leads to the distortion of the fruit (Fig. 1). Damage site of the fruit is distinguished by a characteristic colour pattern, viz., greenish spot in case of purple varieties and a dull green colour tending towards white in varieties

with green fruits. Pupa makes an exit hole and slides into it through which the adult fly emerges. Following the emergence of the fly, the fruit cracks along the inner side of the curve (Fig. 1) exposing the fruit tissues and is rendered unfit for marketing. About 2 and 4% of the fruits were damaged by the midge in the spring seasons of 1973 and 1974 respectively, while the attack was not seen during the later part of 1973. Yield loss due to the pest was of the order of 5% in spring 1974. Earlier, Harris<sup>1</sup> (1938) listed *Asphondylia* sp. as one of the injurious pests of brinjal crop in Tanganyika but did not mention the nature of its damage. The present report forms the first record of the pest on brinjal crop from India.

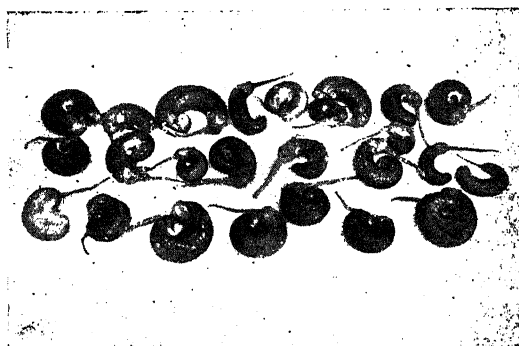


FIG. 1. Damage caused of *Asphondylia* sp.

In spring 1974, observations on the midge incidence were recorded from a chemical control trial conducted for the control of *Leucinodes orbonalis* Guen., wherein the insecticides were sprayed at fortnightly intervals commencing from the first fruit set. The field trial was laid out in randomized block design with three replications on the variety, Dhingra's Multiple Purple.

TABLE I  
Evaluation of insecticides for the control of  
*Asphondylia* sp. on brinjal

| Insecticide     | Dosage<br>Kg<br>ai/ha | Per cent<br>fruit<br>infestation |
|-----------------|-----------------------|----------------------------------|
| Carbaryl        | 1.00                  | 4.12                             |
| Carbaryl        | 2.00                  | 4.08                             |
| Quinalphos      | 0.50                  | 2.78                             |
| Chlorfenvinphos | 0.50                  | 3.21                             |
| Methomyl        | 0.50                  | 3.48                             |
| Pyrethrins      | 0.086                 | 3.49                             |
| Endosulfan      | 0.70                  | 3.13                             |
| Phoixm          | 0.50                  | 3.72                             |
| Control         | ..                    | 3.55                             |
| C.D. (0.05)*    |                       | Not significant                  |

\*Data were statistically analysed after transforming the percentages to arc sin values.

None of the tested insecticides reduced the gall midge incidence significantly, indicating their ineffectiveness in controlling the pest.

Thanks are due to Dr. G. S. Randhawa, Director, for facilities and encouragement and to Dr. Richard, H. Foote, Chief of Systematic Entomology Laboratory, U.S.D.A. (A.R.S.), Beltsville, Maryland, for identification of the pest.

Indian Institute of Horticultural Research,  
255, Upper Palace Orchards,  
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### 'LITTLE LEAF' DISEASE OF GRAPEVINE FROM INDIA

GRAPE (*Vitis vinifera*) is an important commercial crop, extensively cultivated in some parts of India. A number of diseases of fungal origin have been reported from this country but there is no report of any disease caused by virus/mycoplasma-like organisms. However, a number of viral diseases of grapevines have been reported from other grape growing countries<sup>1-4</sup>. Surveys conducted during the year 1973, revealed a disease of grapevine variety 'Merlot Noir' showing 'little leaf' symptoms in the grapevine germplasm collection maintained at the Indian Institute of Horticultural Research, Hessarghatta. The characteristic feature of the disease is smalling of the leaves. The shape and size of the leaves are altered and the leaves become so small and deformed that it becomes difficult to recognize them as grape leaves. The colour of the leaves turn light yellow instead of green and cupping of the leaves was also observed to a certain extent. Internodes are usually close because of the retarded growth of the stem. In early stages, the growth is spindly and tend to become more zigzag at the nodes. The distance between internodes becomes very short and the nodes and internodes are characteristically thin and weak. The general growth of the plant is retarded (Fig. 1). Comparatively very few lateral branches have been observed on the diseased plants. The axillary branches proliferate and produce many secondary branches which are small, thin and weak. No flower and fruit formation has been observed among the infected plants even at the age of 3 years. Roots of the diseased plants have been found to be severely stunted, and produced very few and weak rootlets.

The disease was not transmitted by mechanical sap inoculation to herbaceous hosts though different methods of sap inoculation using different buffers

of different molarities and pH, with or without additives were tried. The disease could be transmitted by grafting or budding of the diseased scion onto the healthy rootstock of the var. Emperor. The symptoms of the disease were observed within 40–50 days following grafting and budding.

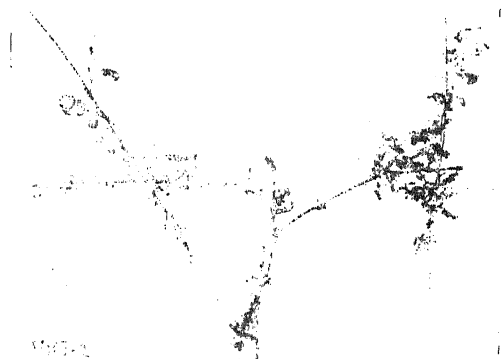


FIG. 1. Grape plant showing little leaf symptoms.

**Heat therapy.**—Diseased cuttings were given hot water treatment at 40, 45, 50 and 55° C. for one hour in a water bath and then planted in pots. The cuttings treated at 45 and 50° C. showed recovery 30–35 days after planting and produced normal leaves but the symptoms reappeared after the lapse of 50–60 days.

**Antibiotic therapy.**—Cuttings (rooted and plain) obtained from diseased plants were treated with 1000 ppm solution of Oxytetracycline hydrochloride for 24 hours and then they were planted in pots for further observation. The treated plants showed recovery symptoms within 25–30 days following antibiotic treatment and produced normal size of leaves and shoots (Fig. 2). The effect of oxytetracycline hydrochloride persisted for about 30–35 days after which the symptoms reappeared which could be suppressed further with another dose of 1000 ppm solution of oxytetracycline hydrochloride.

The causal agent of yellows diseases of plants were thought to be due to viruses. At present, considerable evidence suggests non-viral etiology. Japanese workers<sup>5,6</sup> demonstrated the presence of pleomorphic mycoplasma-like bodies in the case of four different yellows diseases and also reported that tetracycline antibiotics produced remission of the disease in plants infected with mulberry dwarf. On the basis of these findings they proposed that yellows disease agents might be mycoplasma-like organisms rather than viruses. Stoddard<sup>7</sup> and Kenknight<sup>8</sup> suggested a sensitivity of certain yellows diseases to antibiotics and later it was confirmed by

Ishii *et al.*<sup>9</sup>. Now many reports have appeared about the mycoplasma-like bodies in the yellows disease and antibiotic therapy of mycoplasma-like organisms have also been demonstrated<sup>9–13</sup>. Recently Goheen *et al.*<sup>14</sup> reported the association of a Rickettsia-like organisms with Pierce's disease of grapevine and its remission with heat treatment.



FIG. 2

FIG. 2. Plant showing recovery after treating with oxytetracycline hydrochloride antibiotic.

The results obtained from the present studies showed that there is a spontaneous remission of little leaf symptoms when the infected cuttings are subjected to heat as well as to antibiotic treatment, indicating thereby that the grapevine little leaf disease may be due to mycoplasma-like organisms. However, this will be confirmed by further studies.

The authors are highly grateful to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, Bangalore, for providing facilities and encouragement. Thanks are also due to Dr. S. S. Negi, for supplying cuttings of diseased grape plant and also the healthy indicator plants of grapes.

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### EFFECT OF *HOPLOLAIMUS INDICUS* ON GERMINATION OF GREEN GRAM

DURING our investigation on *Hoplolaimus indicus* very poor germination of green gram (*Phaseolus aureus* Roxb.) seed was recorded, in the culture pots having high nematode population. In order to find out the effect of different population levels of this nematode on the germination of green gram this experiment was laid.

Six different populations in log series, of *H. indicus* viz., 0, 10, 100, 1,000, 10,000, 1,00,000 mixed separately in 100 g steamsterilized moistened sandy soil, each contained in 10.0 cm Petri dishes. One hundred surface sterilized (with 0.2% mercuric chloride solution) green gram seeds, variety Pusa Baisakhi, were sown in each dish in five replications. Dishes were kept at random at room temperature (30–35°C) for six days when final germination was counted. All the seeds were taken out of the soil washed cleanly in running tap water and stained in cotton blue-lactophenol solution. Stained seeds were individually teased and examined microscopically in order to see the parasitic behaviour of the nematodes. Data in relation to seed germination is presented in Table I.

No difference in germination at 0 and 10 inoculum level was noticed while with other treatments it differed significantly. Seedlings of 0 and 10 inoculum levels germinated on the 3rd day whereas in 100 and 1,000 levels the germination was delayed by one day. At 10,000 and 1,00,000 levels very poor emergence of seedlings above the soil

was noticed. However, it was observed that a bunch of secondary roots started coming out just above the infected portion of the developing radicle which turned brownish. Occasionally swelling of roots near infected portion was also noticed (Fig. 1).

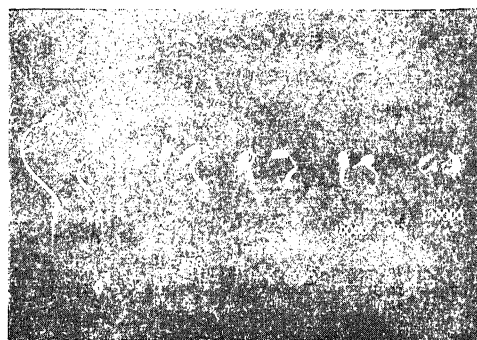


FIG. 1. Effect of different population levels of *H. indicus* on germination of green gram seeds.

TABLE I  
Effect of different population levels of *H. indicus* on germination of green gram

| Sl. No. | Population | Germination percentage<br>Mean of 5 |
|---------|------------|-------------------------------------|
| 1       | 0          | 89.0                                |
| 2       | 10         | 88.2                                |
| 3       | 100        | 63.0                                |
| 4       | 1,000      | 38.6                                |
| 5       | 1,0000     | 11.6                                |
| 6       | 1,00,000   | 2.0                                 |
|         | S.E.       | 1.64                                |
|         | C.D. at 5% | 4.84                                |

Presence of nematode was also observed in between seed coat and cotyledon as well as inside the cotyledon at high inoculum levels. A maximum of 10 nematodes (adult and larvae) were found penetrated in the radicle portion of the just sprouted seeds at 10,000 and 1,00,000 inoculum levels. A few were also noticed in the plumule portion. Although, apparently some injury to the leaf primordia was also observed, but no nematode could be traced in this portion. It appears that nematode attack immediately after sprouting of seeds, retarded their growth prevented their emergence out of the soil and eventually caused death.

It is apparent from the above observations that the nematode population plays a vital role in seed germination if present in soil in high population.

Authors are highly grateful to Dr. S. W. Akhtar, Director, Sugarcane Research Institute, Pusa, Dr. A. P. Mishra, Regional Director, Agricultural

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**RECORD OF A SLUG, *MARIAELLA*  
*DUSSUMIERI* GRAY (ARIOPHANTIDAE :  
BASOMMATOPHORA), AS A NEW PEST  
OF CABBAGE FROM INDIA**

*Mariaella dussumieri* Gray was found damaging the potted cabbage plants during May, 1974 at the Experimental Station of the Indian Institute of Horticultural Research, Hesaraghatta, Bangalore. The slugs inflicted the damage by scraping the epidermis of the leaves and biting holes (Fig. 1).



FIG. 1. Feeding injury by slug.

Mostly the middle whorls of the cabbage head harboured the pest. Slugs were noticed on all the one hundred and fifty plants with an average number of 4 per plant. Apart from direct feeding damage, the pest lowers the market value of the heads by the presence of their excreta. Earlier, Perobrazhenskii (1963) found a grey slug, *Agriolimax agrestis* damaging cabbage at Buryatia Agri. Institute, U.S.S.R. This is the first record of a slug pest on cabbage in India.

Thanks are due to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, Bangalore, for the facilities and to the Director, Zoological Survey of India, Calcutta, for the identification of the pest.

Indian Institute of Horticultural Research,  
255, Upper Palace Orchards,  
Bangalore-6, July 9, 1974.

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**A NOTE ON THE ANDALUSITE DEPOSIT OF  
BAGHISHOTI AREA, DISTRICT MIRZAPUR,  
UTTAR PRADESH**

THE area under study forms the easternmost part of the Bijawar formations of the Pre-Cambrian age lying to the south of the great Vindhyan sedimentary basin of the Son Valley, Uttar Pradesh. It lies on the boundary of Bihar and Uttar Pradesh between the Lats.  $24^{\circ} 20'$  and  $24^{\circ} 25'$  and Longs.  $83^{\circ} 25'$  and  $83^{\circ} 30'$ , and falls under the Survey of India toposheet No. 63 P/7.

Andalusite occurs in the area as porphyroblasts and small crystals (see Fig. 1) in the phyllites and schists. The content of andalusite mineral in the phyllite and schists varies from 5 to 20% and appears to be concentrated on the southern and western margin of the Baghishoti granitic pluton. Crystals are well developed and are grey in colour, prismatic in form with square cross-section, average length is being 1 to 1.5 cm, but crystals as long as 10 to 15 cm are also not uncommon. Average specific gravity of andalusite (of five samples) is 3.05.

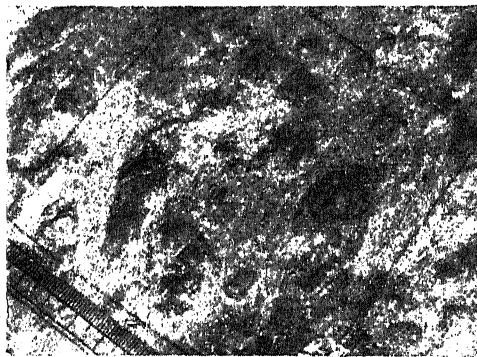


FIG. 1. Showing porphyroblasts of andalusite in the phyllites.

Andalusite bearing rocks occupy a considerable area in the Baghishoti area. During the present investigation only a part of the area has been examined. However, it has been noted that the percentage of the andalusite mineral in the rock appears to be appreciable. From the preliminary examination it appears that a good reserve of andalusite deposit exists. It is, therefore, suggested



that a more detailed investigation be carried out to ascertain the actual reserves of the deposit. The anticipated reserves are of the order of 8 to 10 m tons. In view of the already known andalusite deposit of Dudhi area and the present occurrence of the andalusite deposit of Baghishoti area, leads the author to suggest the possibility of utilisation in a refractory plant at a suitable site in Mirzapur District.

The author is grateful to Dr. R. C. Misra, Professor and Head of the Department of Geology, Lucknow University, for his help and guidance. He is also thankful to the University Grants Commission for the financial assistance in the form of a Senior Research Fellowship.

Department of Geology, A. K. SRIVASTAVA,  
Lucknow University,  
Lucknow 226007. June 24, 1974.

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### EFFECT OF DINITROPHENOL ON GROWTH AND NITROGEN FIXATION IN CLONES OF *CYLINDROSPERMUM MAJUS*

THE role of respiratory ATP in the process of nitrogen fixation in light is not clearly understood. The effects of respiratory inhibitors on nitrogen fixation in blue-green algae have been reported<sup>1-2</sup>. Such studies have been limited due to the lack of specific inhibitors to separate oxidative and photo-synthetic phosphorylations as the thylakoids are thought to be the sites of both photosynthetic and respiratory activities<sup>3</sup>. The aim of the present study is to observe the effect of 2,4-dinitrophenol (DNP) on the growth and nitrogen fixation in various clones of the heterocystous blue-green alga *Cylindrospermum majus*.

The details of the experimental organism and methods are the same as have been described earlier<sup>1</sup>. The clones were treated in 0.01% DNP for 30 min and grown in 15 ml of basal medium. The growth and amounts of nitrogen fixed by the clones were determined after 15 days (Table I). The DNP was found to inhibit both growth and nitrogen fixation to a limited extent. But clones 1 and 3 grew more and clone 3 fixed more nitrogen as compared with the controls, following treatment with DNP. The formation of heterocysts, commonly regarded as sites of nitrogen fixation, was not suppressed by this treatment. Bahal<sup>4</sup> found DNP-stimulated development of heterocysts in

*Anabaena ambigua* while Tyagi<sup>5</sup> observed the inhibition of heterocyst formation by DNP at a concentration of  $5 \times 10^{-4}$  M in *Anabaena doliolum* and suggested that it was probably due to a block in the supply of respiratory ATP.

TABLE I

Optical density and cell nitrogen of various clones of *Cylindrospermum majus* (Average values of two replicates)

| Clone No. | Control       |             | DNP treatment |             |
|-----------|---------------|-------------|---------------|-------------|
|           | Growth (O.D.) | Cell N (mg) | Growth (O.D.) | Cell N (mg) |
| 1         | 0.067         | 0.364       | 0.092         | 0.336       |
| 2         | 0.097         | 0.392       | 0.090         | 0.392       |
| 3         | 0.090         | 0.301       | 0.102         | 0.462       |
| 4         | 0.127         | 0.497       | 0.092         | 0.476       |
| 5         | 0.122         | 0.567       | 0.100         | 0.434       |
| 6         | 0.122         | 0.406       | 0.057         | 0.322       |

The statistical analysis of variance revealed that neither the control nor the DNP-treated clones showed any significant variation in nitrogen fixation. These results indicate that in the process of nitrogen fixation in light, ATP produced from the oxidative phosphorylation is not needed. The requirements of energy and carbon skeletons are fulfilled mainly through the process of photosynthesis. The oxidative phosphorylation may supply ATP only when nitrogen fixation occurs under dark conditions. Besides, ATP produced on breakdown of polyphosphates<sup>6</sup>, which are abundant in blue-green algae<sup>7-10</sup>, may also be a source of energy in these organisms.

The author is thankful to Professors H. D. Kumar and Y. D. Tiagi for guidance.

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Udaipur 313001, India, July 3, 1974.

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## SHORT SCIENTIFIC NOTES

### Vitamin C Content in *Moringa* Pod Vegetable

The tender pod-like fruits of *Moringa* tree are used in the preparation of various types of vegetable-curries and pickles throughout India. The pods start ripening during the last week of March and are then cooked alongwith "Sambar".

In our studies on the genetical improvement of *Moringa* for wood-pulp we came across extensive variation in the taste of pods of *M. oleifera* and *M. concanensis* which varied from very sweet to extremely bitter. Different grades of sweetness of both raw and cooked pods of *M. oleifera* was the basis for selection of clones 3, 6, 7 and 10 from vegetatively propagated trees planted under standard nursery environment. From amongst the four clones of *M. oleifera*, clones 6 and 7 flower almost throughout the year and they, therefore, fall in the category of "Baramasi" varieties. Both leaves and fruit-juice of *M. oleifera* are known to be rich in vitamin C content<sup>1-4</sup>.

The fresh pods of the four sweet clones of *M. oleifera* and of one bitter clone of *M. concanensis* were screened for ascorbic acid content. The fruits from each tree were also collected randomly during II week of April and a composite sample of 10 g was ground with 12% oxalin acid. Ascorbic acid content was then determined by titremetric method using 2-6 dichlorophenol indophenol. The results are given in Table I. *M. concanensis* had the highest ascorbic acid content in the bitter fruit of the clone evaluated.

TABLE I

| Sl. No.                                     | <i>M. oleifera</i> |        |       | <i>M. concanensis</i> |        |
|---|--------------------|--------|-------|-----------------------|--------|
|   | 7                  | 3      | 10    | 6                     | 1      |
| Baramasi Long flowering and fruiting period | +                  | —      | —     | +                     | —      |
| Fruit size large                            | +                  | +      | +     | —                     | +      |
| Fruit very sweet                            | +                  | +      | +     | +                     | —      |
| Fruit very bitter                           | —                  | —      | —     | —                     | +      |
| Ascorbic acid content mg per 100 gm pulp    | 126.41             | 124.53 | 97.17 | 91.51                 | 132.17 |

+ Present; — Absent;

Amongst the sweet clones of *M. oleifera* clones 7 and 3 had the highest amount of ascorbic acid followed by 10 and 6. Clone 6 although sweet has a poor yield in spite of its long flowering period due to sterility. Work is also in progress in this

Laboratory on screening of new germplasm of *M. oleifera* for other important nutritive contents such as proteins, carbohydrates and other vitamins.

We are grateful to professor T. S. Sadasivan, for suggesting the problem of screening the sweet clones of *M. oleifera* for vitamin C content.

Tree Genetics Laboratory, P. D. DOGRA.  
National Botanic Gardens (CSIR), B. P. SINGH.  
Lucknow, June 10, 1974. S. TANDON.

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### Comparative Observations on Glume, Spikelet and Stomatal Length in Haploid, Diploid and Tetraploid Rices

Spontaneous occurrence of tetraploids in the  $F_2$  generation of two diploid rice varieties, viz., J.B.S. 820 and AC. 1225 has been reported<sup>1</sup>. AC. 1225 has long sterile glume while in J.B.S. 820 the sterile glumes are asymmetrical in length. One haploid plant was later isolated from the  $F_2$  population. An attempt was made to study the ploidy effect on the glume (sterile lemma), spikelet and stomatal length of the cytotypes.

The haploid plant, diploid parents (J.B.S. 820 and AC. 1225) and a typical tetraploid Tet-6, were studied. All the cytotypes had asymmetrical glumes and therefore, glumes on lemma and palea sides were separately observed. Stomata of dorsal side of the second leaves from top were always selected. One hundred measurements were taken in all cases.

Data are presented in Table I along with the range and mean.

There is a linear increase in the mean length of spikelet, glume and stomata from haploidy to tetraploidy. The proportion of increase is, however, not the same in all characters. The mean spikelet length of diploids was approximately twice the mean length of haploid while the increase was marginal from diploidy to tetraploidy. A similar trend was noted in the case of glume length on the palea side; there was overlapping of measurements. The ploidy effect was thus most pronounced in the length of lemma side glume.

TABLE I  
Spikelet, glume and stomatal length in cytotypes of rice

| Charac-<br>ters | Spikelet length in mm |                 | Glume length in mm |                 |            |                 | Stomatal length in $\mu$ |                 |
|-----------------|-----------------------|-----------------|--------------------|-----------------|------------|-----------------|--------------------------|-----------------|
|                 |                       |                 | Palea side         |                 | Lemma side |                 |                          |                 |
|                 | Types                 | Range           | Mean               | Range           | Mean       | Range           | Mean                     | Range           |
| Haploid         | 3.50-4.50             | 4.01 $\pm$ 0.07 | 0.50-3.90          | 2.30 $\pm$ 0.17 | 0.50-2.00  | 0.97 $\pm$ 0.12 | 4.50-7.00                | 5.64 $\pm$ 0.07 |
| J.B.S. 820      | 6.98-8.60             | 7.77 $\pm$ 0.05 | 3.00-7.00          | 5.42 $\pm$ 0.14 | 2.10-6.00  | 3.97 $\pm$ 0.14 | 5.50-9.10                | 6.68 $\pm$ 0.09 |
| AC. 1225        | 7.30-9.00             | 8.36 $\pm$ 0.06 | 4.70-8.60          | 6.56 $\pm$ 0.28 | 3.80-9.60  | 5.05 $\pm$ 0.18 | 6.20-8.00                | 6.63 $\pm$ 0.06 |
| Tet-6           | 9.07-10.54            | 9.91 $\pm$ 0.02 | 5.00-13.00         | 7.88 $\pm$ 0.21 | 5.00-10.00 | 6.68 $\pm$ 0.19 | 7.10-10.00               | 8.44 $\pm$ 0.11 |

The linear increase in the mean stomatal length from haploidy to diploidy and from diploidy to tetraploidy was also marginal with overlapping of measurements.

Rajendra Agricultural Univ.,  
Agricultural Research Institute,  
Sabour, Bhagalpur, July 3, 1974.

R. THAKUR.

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#### An *Alternaria* Leaf Spot of Tapioca

Tapioca plants (*Manihot esculenta* Crantz), aged, about 7 months, in the instructional farm of the College of Agriculture, Vellayani, were affected by a leaf spot disease during April 1974. The spots were observed mainly near the tips of the lobes of the leaf. They were minute, scattered with whitish grey centre and brown margin. Often the leaf tips dried due to the formation of a number of spots. Rarely, spots were also observed on the basal part of the leaf blade. Repeated isolations from the infected regions yielded an *Alternaria* species. The pathogenicity of the same was established by artificially spray inoculating tapioca plants with 6 day old culture of the organism. Typical leaf spot symptoms were formed in 7-10 days after inoculation. The fungus was reisolated into pure culture from inoculated plants.

A detailed study of the morphological characters of the fungus on potato-dextrose agar revealed the following characters. The mycelium was slightly dark and 2.25-10.5  $\mu$  wide. Conidia mostly single, sometimes in chains of 1-3, provided with 1-9 transverse septa and a maximum of 3 longitudinal septa. Conidia were dark brown, obclavate, rarely elliptical, attenuate; beak short, rarely long. Including the beak the conidia measured 14.04-76.38  $\mu$  (40.07  $\mu$ ) the beak alone was 8.13-61.75  $\mu$  in length (15.37  $\mu$ ). The breadth at the basal region ranged between 5.68  $\mu$ -13.0  $\mu$  (10.75  $\mu$ ) and

at the base of the beak it was 3.25  $\mu$ -8.12  $\mu$ . The conidiophore measured on the average 78.5  $\mu$   $\times$  3.25  $\mu$  with 6-9 septations and 1 or 2 lateral scars.

The morphological details closely resembled these of *Alternaria palandui* Ayyangar, reported from *Allium* species by Ayyangar<sup>1</sup> (1928). Artificial cross inoculation of *Allium cepa* L. by the isolate from tapioca gave positive results. From the close resemblance of the morphological characters and by the cross inoculation tests carried out, the isolate from tapioca was identified as *A. palandui* Ayyangar. Tapioca is recorded for the first time as a host of this organism causing leaf blight disease.

Division of Plant  
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July 4, 1974.

P. K. SATHYARAJAN.  
M. CHANDRASEKHARAN NAIR.  
M. RAMANATHA MENON.

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#### Occurrence of White Grubs in Haryana

A serious incidence of white grub on *bajra* (*Pennisetum typhoideum*) and groundnut was observed during 1971 in village Mandhana (District Mohindergarh). During October, 1971 one square metre area in a harvested *bajra* field was carefully dug which contained, at depths varying from 30 to 96 cm, 23 adults, 3 pupae and 2 first instar grubs of *Lachnosterna fissa* Brenske.

A severe insect damage to newly sprouted *ber* (*Ziziphus mauritiana* Lmk.), leaves was reported from Kankraula (District Gurgaon) in the first week of July, 1972. 27 adult beetles were collected from upper 15 cm sandy-soil layer of the orchard and these represented 4 species of white grubs, viz., *Holotrichia insularis* Bren. (21 specimens), *Anomala ruficapilla* Burm. (3 specimens),

*A. dorsalis* F. var *fraterna* Burm. (2 specimens), and *A. bengalensis* Bl. (1 specimen).

Peach tree leaves at Gurgaon were severely damaged by some insect during July, 1972. Spraying of a medium sized tree with endrin 0.02% emulsion at sun-set knocked down 60 adults of white grub, *Holotrichia* sp. nr. *problematica* Bren. which were collected next morning.

These reports indicate that several species of Scarabaeid white grubs are becoming serious pests of *kharif* crops as well as fruit trees in Haryana.

Out of the white grub species mentioned in this communication, *H. insularis* and *A. bengalensis*

have earlier been reported to damage sorghum, bajra, sugarcane, chillies, groundnut, suna-hemp, brinjal, cucurbits, ladys' finger and cowpeas in Rajasthan (Srivastava and Khan, 1963)<sup>1</sup>.

Thanks are due to Dr. P. K. Sen-Sarma, Forest Research Institute, Dehra Dun, for identifying the insects.

Department of Entomology, A. N. VERMA.  
Haryana Agricultural University,  
Hissar, June 4, 1974.

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## REVIEWS AND NOTICES OF BOOKS

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Annual Review of Fluid Mechanics (Vol. 4). By M. V. Dyke, W. G. Vincenti and J. V. Wehausen. (Annual Reviews Inc., 4139 El Camino Way, Palo Alto, Calif., Calif. 94306), 1972. Pp. ix + 504. Price \$ 12.00 (U.S.A.), \$ 12.50 (Elsewhere).

Within the short period of a few years since the commencement of publication of these annual reviews, they have earned a permanent and respected place in the literature of fluid mechanics. Volume 4 maintains the high standards set by its predecessors, and offers rich and diverse fare which will be both interesting and instructive to fluid dynamicists. Among the subjects covered, about a third is of direct interest to engineers (heat disposal from power generation, chemically reacting flows, vortex breakdown, cavity and wake flows, wing-body interaction in aircraft); another third is geophysical or environmental (mantle convection, rotating and stratified flows, magnetohydrodynamics of the earth's core, self-gravitating gaseous disks, oil spreading on sea). There are several articles of general interest also (self-similar solutions, periodic flows, liquids containing bubbles, sailing, bounds on flow quantities), and one, on the locomotion of protozoa.

Experimental workers will probably feel that there are too few articles for them in this volume as in previous ones, but by and large the Review will be found to be a valuable survey of some of the most interesting recent investigations in fluid mechanics.

It would be very useful to have this series in paperback, so that more workers could possess their own copies, as I am sure many would like to.

R. NARASIMHA.

Quantum Mechanics in a New Key. By Alfred Lande—Exposition Press, 1973 (Exposition Press, Inc., 50 Jericho Turnpike, Jericho, New York 11753), 1973. Pp. x + 131. Price \$ 6.50.

This book is the third in a series which represent the efforts of the author, a renowned physicist, to clarify the foundations of quantum mechanics and demystify the notions of complementarity, duality, etc., which are so prominent in usual treatments. He attempts to provide a well motivated derivation of the usual computational rules which involve superposable vectors in a complex Hilbert space and operators on these representing physical quantities. The starting point of this derivation is a set of non quantum postulates, governing a probabilistic scheme of description of the results of measurements. This is the part of the book I found most interesting chapters II to V—even though the argument does seem after the fact at points. The first chapter is meant to provide a negative motivation for the rest and consists of an attack on the usual presentations. I for one failed to understand the covariance objection to Bragg reflection and the oscillator dilemma, both of which are intended to discredit the usual interpretation. One cannot help feeling that the author deliberately sets up a very naive argument, the better to demolish it later. The treatment of the Einstein Podolsky Rosen paradox—page 111—is unique in that it simply denies the existence of correlations in the results of measurements on two spatially separated parts of a system which had been together in the past. This would have surprised the authors of the paradox as much as those who tried to rebut their viewpoint!

In view of the many inaccuracies and distortions, I do not think the claims of pedagogic value made

for the book are justified. Specialists in the foundations of quantum theory may, however, feel like taking a look. In the case of at least one to whom I showed the book, the look was not a long one!

RAJARAM NITYANANDA.

**Pharmacognosy of Powdered Crude Drugs.** By M. A. Iyengar. Pp. xiii + 63. Price Rs. 15; **A Hand Book of Pharmacology.** By M. A. Iyengar. Pp. 98. Price Rs. 15.00. (Published by the Author. College of Pharmacy, Kasturba Medical College, Manipal-576119), 1974.

The technical education has made tremendous progress in recent years. One of the major drawbacks to cope up with the progress is non availability of the text books. The text books on a subject like Pharmacognosy are very few and most of them are written by foreign authors. Dr. M. A. Iyengar has succeeded in publishing two books in Pharmacognosy, namely *A Hand Book of Pharmacognosy* and *Pharmacognosy of the Powdered Crude Drugs*.

*A Hand Book of Pharmacognosy* mainly deals with the basic knowledge pertaining to the official source, family, major chemical constituents and their tests, the Pharmacological and therapeutical uses of about eighty crude drugs. The author has taken pains to describe the microscopic characteristics of some of the powdered crude drugs commonly used, in his book *Pharmacognosy of Powdered Crude Drugs*. The author has maintained the originality in describing the diagrammatic features of these drugs.

These two books are very useful for the para medical students particularly to those who undergo Pharmacy course. The *Hand Book of Pharmacognosy* is particularly useful to the students undergoing the course in Diploma in Pharmacy.

V. B. DESAI.

## ANNOUNCEMENTS

### Award of Research Degrees

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Mathematics to Shri Brajakishore for his thesis entitled "Summability of Fourier Series and Allied Problems"; Ph.D. degree in Chemistry to Shri Santosh Kumar Mahapatra for his thesis entitled "Studies of Heterocyclic Compounds". Ph.D. degree in Science (Zoology) to Shri Murari Mohan Dash for his thesis entitled "Studies on the the Population and Biometry of the Potato Aphid,

*Myzus* (Necterosiphon) *persicae* Sulzer (Aphididae-Homoptera, Insecta)".

The M.S. University of Baroda has awarded the Ph.D. degree in Botany to Shri Dharmendrakumar Natwarlal Thaker for his thesis entitled "Floristic and Ethnobotanical Studies on Kawant range forests in Central Gujarat"; Ph.D. degree in Psychology to Shrimati Siddhida Jahardan Mehta for her thesis entitled "An investigation into the Effectiveness of Programmed Material in English for developing Reading Ability".

Karnatak University, Dharwar, has awarded the Ph.D. degree in Chemistry to Shri Goudar Timmanagoud Ramanagoud for his thesis entitled "Physico-Chemical Investigations on Complexes of Some Transition Metals".

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Shri Syed Ghouse Peeran for his thesis entitled "Synthesis and spectral studies of Cis (Z) and Trans (E) Unsaturated Sulphide—Sulphones and Disulphides"; Ph.D. degree in Physics to Shri B. P. Nagireddy, Shri S. Mohan and Shri J. Lakshman Rao for their theses entitled "Studies in Low Temperatures—Elastic and Thermal Properties of Some Ferrites", "Studies in Solid State Physics Effect of Temperature of Electrical Conductivity and Hall Effect in Thin Films of Silver and Copper" and "Studies on the Optical Absorption spectra of some first group transition metal ions in certain Inorganic and Organic Crystals" respectively.

### Books Received

*Annual Review of Nuclear Science* (X)

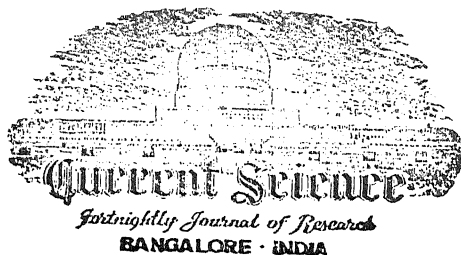
Edited by Emilio Segre, J. Robb Gr. H. Pierre Noyes. (Annual Reviews, Inc., Caminoway, Palo Alto, California 94306 Pp. 449. Price: U.S.A., \$12.00.; Elsewhere \$12.50.

*Annual Review of Physical Chemistry* (Vol.

Edited by H. Eyring, C. J. Christensen and H. Johnston. (Annual Reviews, Inc., Palo Alto, California 94306). Pp. 546. Price: U.S.A. \$12.00; Elsewhere \$12.50.

*Dictionary of Water and Water Engineering.* By A. Nelson and K. D. Nelson. (Butterworth Group, 88, Kingsway, London WC 2 B 6 A B), 1973. Pp. vi + 271. Price £3.60.

*Careers in Industrial Research and Development.* By J. H. Saunders. (Marcel Dekker, Inc., 95, Madison Avenue, New York, N.Y. 10016), 1974. Pp. xi + 254. Price: \$11.75; prepaid student Price \$7.75.



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## ABSTRACT

The need for modifying the potential function  $V(\psi)$  corresponding to the rotation about the bond  $C^\alpha-C$  in peptides and polypeptides, from three-fold minima and a low barrier, to  $V(\psi) = 2.0 (1 - \cos 2\psi)$  is stressed. Taking the new expression for  $V(\psi)$  it is shown that the theory for a pair of peptides is in good agreement with the conformational data obtained from the crystal structures of peptides and proteins. Applications of the new form of  $V(\psi)$  to LL and LD bends in peptides and proteins also improve the agreement between theory and observation.

## INTRODUCTION

IN connection with the calculations of the energy of a dipeptide unit corresponding to the conformation  $(\phi, \psi)$ , suitable potential functions for various types of interactions have been used by different laboratories. Although differing in minor details, all of them are essentially similar<sup>1-4</sup>. Considering in particular, the potential functions associated with the torsion angles  $\phi$  and  $\psi$ , the functions in common use have the following forms:

$$V(\phi) = \frac{1}{2} V_\phi (1 \pm \cos 3\phi) \quad (1)$$

with  $V_\phi$  varying between 0.6 and 1.5 kcal/mole; and

$$V(\psi) = \frac{1}{2} V_\psi (1 - \cos 3\psi) \quad (2)$$

with  $V(\psi)$  having values between 0.25 and 1.0 kcal/mole.

As a justification for using a low barrier for  $V(\psi)$ , it is stated that the barrier to the internal rotation about the  $C^\alpha-C$  bond in compounds of the type  $CH_3C(X)O$  ( $X = H, F, Cl, Br, N, OH$ ) is found to be very small and having three-fold minima<sup>5</sup>. However, for the rotation  $\psi$  about the bond  $C^\alpha-C$  in the peptide group, in which the nitrogen atom is attached to the C-atom on one side and to the  $C^\alpha$ -atom on the other side, as shown in Fig. 1, the nature and height of the barrier could be different. A recent analysis of the distribution of observed conformations in small peptides and in the non-helical regions of proteins indicated that the barrier to rotation about the bond  $C^\alpha-C$  is not three-fold<sup>6</sup>. These observed conformations are found to cluster within  $\pm 30^\circ$  around

$\psi = 0^\circ$  and  $\psi = 180^\circ$ , corresponding approximately to the *trans* and *cis* positions of the nitrogen atoms, as shown in Fig. 2<sup>6</sup>. Infrared studies of amides made by Shimanouchi<sup>7</sup> also indicated that the stable conformations that occur corresponded to either  $\psi = 0^\circ$ , or  $180^\circ$ . These observations suggest that the potential  $V(\psi)$ , for rotation about the bond  $C^\alpha-C$ , can be of the form,

$$V(\psi) = \frac{1}{2} V_\psi (1 - \cos 2\psi) \quad (3)$$

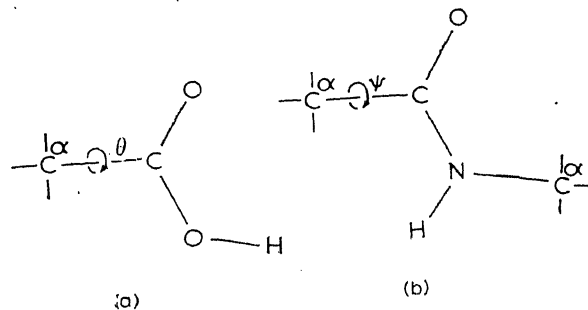


FIG. 1. Diagrams illustrating the differences in geometry related to the rotation about the bond  $C^\alpha-C$ , in the case of (a) a carboxylic acid and (b) a peptide.

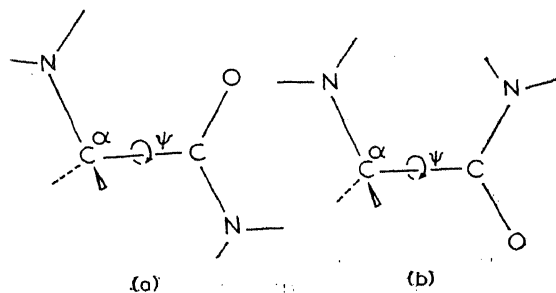


FIG. 2. (a) and (b) show respectively, the *trans* and *cis* positions of the nitrogen atoms in a dipeptide.

In view of this, preliminary quantum chemical calculations, using an *ab-initio* method, were carried out by us sometime ago on a model compound  $NH_2CH_2CONH_2$ . For  $\psi = 90^\circ$ , the total energy

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was found to be more by as much as 6.0 kcal/mole for  $\psi$  near  $90^\circ$  as compared to the energy at  $\psi = 0^\circ$  or  $180^\circ$ . Another set of calculations using IEHT method developed by Rein *et al.*<sup>8</sup> and using the Cusach approximation, on the same model compound, indicated that the total energy variation with respect to  $\psi$  has minima at  $\psi = 0^\circ$  and  $\psi = 180^\circ$  with a maximum at  $\psi = 90^\circ$ . The total energy difference between maxima and minima was found to be of the order of 4.5 kcal/mole. Recent calculations of Pople and Radom<sup>9</sup>, for the same model compound using an *ab-initio* procedure, have indicated minima at  $\psi = 0^\circ$  and  $\psi = 180^\circ$ , for  $\phi = 90^\circ$  (since the calculations have been done only at intervals of  $60^\circ$ , no precise value for maxima, and the difference between maximum and minimum energy, could be obtained). In performing these calculations, the total energy was first minimized with respect to  $\phi$ , and then with respect to  $\psi$ . Therefore, the total energy variation with respect to  $\psi$  which gives minima at  $\psi = 0^\circ$  and  $\psi = 180^\circ$  is indicative of the fact that the form of the internal rotational potential function  $V(\psi)$  around C—C bond has two-fold minima. These quantum-chemical calculations also suggest that the barrier to the internal rotation about C $^\alpha$ —C bond has a large value compared to the currently accepted value of 1.0 kcal/mole or less. Though the quantum-chemical calculations give the total energy difference, the contributions from other interactions in this range is expected to vary little and therefore, the barrier to internal rotation about the bond C $^\alpha$ —C is taken to be as large as 4.0 kcal/mole.

#### APPLICATION OF THE MODIFIED $\psi$ -POTENTIAL

The potential energy variation, for a pair of linked peptide units having a C $^\beta$ -atom, using the 6-exp non-bonded potential function, the electrostatic interaction energy as given in Ref. 1 and the torsional potential  $V(\psi)$  as

$$V(\psi) = 2.0(1 - \cos 2\psi) \quad (4)$$

is shown in Fig. 3 (b). In view of our recent quantum chemical calculations made on simple amides which indicated that the barrier height to the internal rotation around the N—C $^\alpha$  bond is very small, we have neglected the term  $V(\phi)$ . The hydrogen bond energy contribution is also not included. The convention used for drawing the potential energy maps is as suggested by IUPAC-IUB Commission<sup>11</sup>, and the dihedral angles ( $\phi, \psi$ ) are defined as given in their recommendation. By making the only change

$$V(\psi) = 0.25(1 - \cos 3\psi) \quad (5)$$

the variation in energy is shown in Fig. 3 (a). The main difference between Fig. 3 (a) and Fig. 3 (b) is that the region between  $\psi = 60^\circ$  and  $120^\circ$  is

practically forbidden for all conformations of an alanine dipeptide unit according to the new  $\psi$ -potential, whereas it is not so with the old potential. This is because of the fact that the  $\psi$ -potential rises by 4.0 kcal/mole for  $\psi = 90^\circ$ .

In Fig. 3 (b) are also plotted the ( $\phi, \psi$ ) values for non-glycyl linear oligo-peptides (tri-, tetra- and hexa-peptides) as well as cyclic peptides. These points lie in the low energy regions of the map. The low energy regions shown in Fig. 3 (b) thus become the allowed conformations for a dipeptide. In a polypeptide chain, though some of the low energy regions of a pair of peptide unit may become disallowed, it is obvious that the disallowed conformations for dipeptide unit will not be a low energy conformation for a polypeptide chain. Thus, if a comparison of Fig. 3 (a) or 3 (b) with the empirical plot obtained from the protein data of Pohl<sup>10</sup>, and shown in Fig. 3 (c) (to facilitate ready comparison) is made, much better agreement is obtained for Fig. 3 (b) than for Fig. 3 (a). However, while making the comparison of Fig. 3 (c) with either Fig. 3 (a) or 3 (b), it should be remembered that the  $\alpha$ -helical type of conformations near ( $-60^\circ, \pm 60^\circ$ ) and the  $\beta$ -structure conformations have been included in Pohl's diagram, although they are not particularly favoured for a dipeptide conformation. This explains the essential difference between Fig. 3 (b) and Fig. 3 (c).

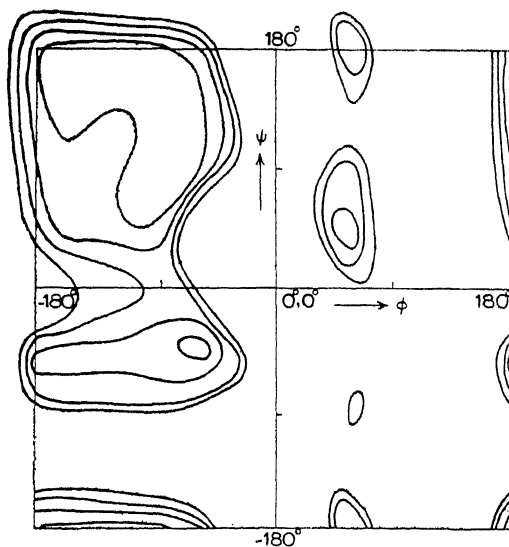


Fig. 3 (a). Isoenergy curves for a pair of peptide units linked at C $^\alpha$  ( $V_{tot} = V_{nb} + V_{es} + V(\phi)$ ) at intervals of 1.0 kcal/mole, for an alanyl dipeptide, with  $V(\psi)$  having three-fold minima at  $-180^\circ$ ,  $-60^\circ$  and  $+60^\circ$  and a small barrier of 0.5 kcal/mole.

In all our calculations, we have considered the geometry for the peptide unit having  $\tau(C^\alpha-C-O) = 115.6^\circ$  and  $\tau(N-C_2^\alpha-C) = 112.5^\circ$ .

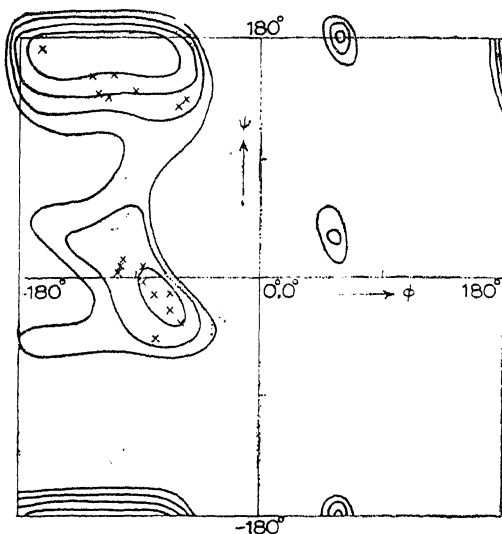


FIG. 3 (b). A similar diagram for an alanyl dipeptide unit, but with  $V(\psi)$  having two-fold minima at  $\psi = 0^\circ$  and  $180^\circ$  and a barrier of 4.0 kcal/mole in between them at  $\psi = 90^\circ$ . The values of  $(\phi, \psi)$  as obtained from crystal structure data of small peptides for non-glycyl residues are shown by cross (X).

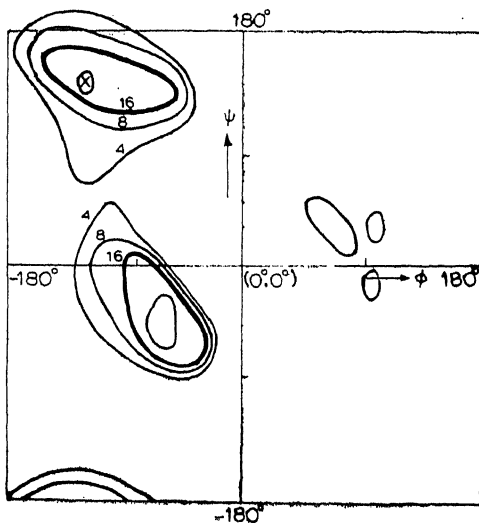


FIG. 3. (c) Isoprobability curves in the  $(\phi, \psi)$ -plane, reported by Pohl<sup>10</sup> (including glycyl examples). The curves at intervals of factors of 2. Note the similarity between Figs. (b) and (c).

The energy for a glycyl dipeptide unit, using the new potential function for  $V(\psi)$  is shown in Fig. 3 (d). In Fig. 3 (d) are also plotted the

$(\phi, \psi)$  values for linear oligopeptides and cyclic peptides for glycyl residues along with the  $(\phi, \psi)$  values for glycyl residues in the non-helical regions of lysozyme, myoglobin and chymotrypsin as obtained from crystal structure data. The distribution of points clearly shows that the energy map drawn by using the new potential function for  $V(\psi)$  is in much better agreement with the observed data than the energy maps reported so far. Similarly, if the isoenergy contours of Fig. 3 (d) are compared with the iso-probability curves given by Pohl<sup>10</sup> for glycyl residues (not shown here), as obtained from protein crystal data, the agreement is found to be excellent.

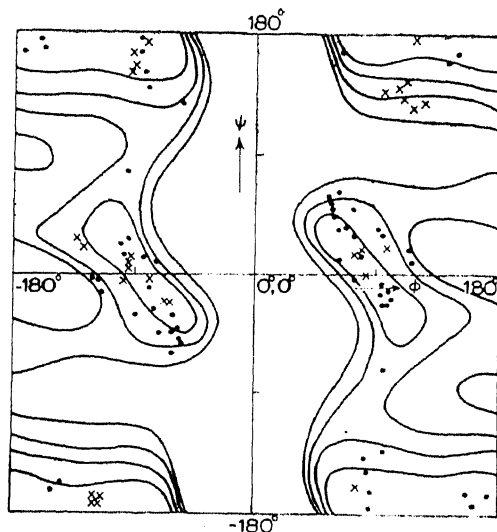


FIG. 3 (d). Diagram similar to (b), but for a glycyl dipeptide unit, using  $V(\psi)$  having two-fold minima and a barrier of 4.0 kcal/mole. The values of  $(\phi, \psi)$  for glycyl residues only, in peptides and proteins are plotted. X—denotes data from peptides; •—denotes data from lysozyme, chymotrypsin and myoglobin.

#### APPLICATION TO BETA-BENDS

The theory of these bends was published by Chandrasekaran *et al.*<sup>13</sup>. This theory predicted minima for an LL bend for values of  $(\phi_2, \psi_2)$ ,  $(\phi_3, \psi_3)$  close to  $(-50^\circ, -50^\circ)$ ;  $(-110^\circ, 40^\circ)$  and to  $(-60^\circ, 100^\circ)$ ;  $(60^\circ, 40^\circ)$ . Thus,  $|\psi_3|$  was always greater than  $30^\circ$ . However Table II and Table III of Ref. 13 show that, for most of the observed conformation, the value of  $|\psi_3|$  lies between  $0^\circ$  and  $20^\circ$ . The mean of  $|\psi_3|$  for observed data as obtained from these tables of Ref. 13 in lysozyme and chymotrypsin is  $14^\circ$  and for small peptides is  $9^\circ$ , thus leading to a discrepancy between theory and observation. When the calculations were repeated using the new potential

$V(\psi)$ , the minimum energy conformations of the LL bend come close to  $(-60^\circ, -30^\circ)$ ,  $(-90^\circ, 20^\circ)$  and  $(-60^\circ, 140^\circ)$ ;  $(60^\circ, 10^\circ)$ , agreeing well with the observations.

Thus, the data presented in this note indicate that a form of the  $\psi$ -potential with two-fold minima and a relatively high barrier of 4.0 kcal/mole is the one that may have to be adopted for the classical energy calculations.

#### ACKNOWLEDGEMENT

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### ALL-INDIA SYMPOSIUM ON APPLIED OPTICS AND EXHIBITION OF OPTICAL INSTRUMENTS, BANGALORE

**A**n all-India Symposium on Applied Optics and Exhibition of Optical Instruments were held during 28-30 November, 1974, at the Indian Institute of Science, Bangalore.

The symposium was convened under the joint auspices of the Central Instruments and Services Laboratory, Centre for Information Processing and Department of Physics, Indian Institute of Science. Profs. M. Ramakrishna Rao, S. V. Pappu and P. S. Narayanan were the conveners. About 100 delegates from all over India participated in the symposium.

The proceedings of the three-day symposium began with the inaugural function. Prof. S. Bhagavantam, former Scientific Adviser to Minister for Defence, delivered the inaugural address. Prof. S. Dhawan, the Director of the Institute, apprised the gathering of the various activities of the Institute in the field of Applied Optics. Sri. S. M. Krishna, Minister for Industries, Karnataka State, declared open the exhibition and released the Souvenir.

Prof. H. Narasimhaiah, Vice-Chancellor of Bangalore University, presided over the inaugural function.

About forty papers were presented in five technical sessions under the following headings: Lasers and Applications; Optical Processing and Holography; Optical Thin Films and Materials; Optical Measurements and Testing; Optical Instruments and Devices.

An exhibition of various optical instruments manufactured in India was held along with the symposium. About twenty companies participated in the exhibition, bringing their products to the attention of the scientists and engineers working in the field of Applied Optics.

Prior to the symposium a four-week winter school in optical engineering was organised for college teachers under the education program. About twenty teachers and scientists from colleges and research organisations attended the winter school.

# EVALUATION OF NUMERICAL EVIDENCE IN FORENSIC COMPARISON ANALYSES CRITERIA FOR IDENTITY AND ASSESSMENT OF EVIDENTIAL VALUE

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Forensic Science Laboratory, Byculla, Bombay-8

## ABSTRACT

Two alternative methods of evaluating a discrepancy index are discussed, with a view to match crime/clue material with a comparison/source sample. One is put as  $\theta = \sum_{i=1}^N (Q_i - 1)^2/q_i^2$  (cf. Parker's C) and another,  $\theta = \frac{1}{N} \sum_{i=1}^N Q_i (1 + t_i q_i)$  based on the discrepancy quotient Q and the overall coefficient of variation q. A simple evaluation of the uniqueness of a crime material is described on the basis of attributes measured in the source and the population, their mean values and the respective standard deviations.

**F**ORENSIC comparison analyses are undertaken to determine if a "crime sample" has originated from an alleged source. The inference drawn from non-numerical analyses like tool marks examination, e.g., in forensic ballistics or hand-in-glove fit of a crime sample with its remnant available elsewhere, can (when good characteristic matches are observed) be interpreted directly without "any reasonable doubt". This however is not possible in (numerical) material analyses<sup>1</sup>. In the former the characteristic match is obvious, but in the latter one has to cautiously interpret the results. Forensic analytical data have been evaluated by Parker<sup>2</sup> (human hair), Hoffman<sup>3</sup> (soils) and Lawson and Framan<sup>4</sup> (general).

Evaluation of analytical data in forensic problems is considered here firstly to establish if the crime and comparison (alleged source) samples match and secondly, that if these do so, then what proportion of the population of such material in question attain the levels of the attributes analysed. The lower it is, the more weighty is the evidence.

## 1. CRITERIA FOR IDENTITY

For matching or otherwise of a crime sample with the source, Parker<sup>2</sup> defined the discrepancy index, C, as:

$$C = \sum_{i=1}^N D_i^2 = \sum_{i=1}^N (X_i - Y_i)^2/\lambda_i^2 \quad (1)$$

where  $X_i$  and  $Y_i$  are the values for the  $i$ -th attribute measured in the crime sample and the alleged source respectively.  $\lambda_i$  is the combined standard deviation resulting from the standard deviation  $\delta_1$  in the crime sample, due to the measurement errors and the standard deviation  $\delta_2$  in the source, mainly due to the intrinsic variability;  $\lambda^2 = \delta_1^2 + \delta_2^2$ . If N uncorrelated attributes are measured, C is distributed as  $\chi^2$  with N degrees of freedom, if the crime sample originates from the alleged source. The observed C may be compared with the critical value for C, viz.,  $C_0$ , from the statistical  $\chi^2$  tables. At a given chosen probability value, say 0.01 (Parker),

if  $C < C_0$  then the crime sample could stem from the alleged source, and if in fact it does, the probability of finding  $C > C_0$  is as low as 0.01.

An alternative to Parker's C is described here, based on discrepancy quotient (*vide infra*) which is significant to the forensic analyst. Moreover, Parker used  $\ln c$  as against  $c$  (concentration); this is laborious, without making any significant difference in the results using  $c$  conveniently.

Let the values of a given attribute in the crime sample and the alleged source be:  $X_1 \pm \delta_1$  and  $X_2 \pm \delta_2$  respectively, and let  $\delta_1/X_1 = q_1$  and  $\delta_2/X_2 = q_2$ . We define discrepancy quotient, Q, as  $Q = X_1/X_2$ , we have  $(X_1 \pm \delta_1)/(X_2 \pm \delta_2) = Q \pm k$ , where  $k/Q = \sqrt{q_1^2 + q_2^2} = q$  (let). Parker's element of discrepancy index, viz.,  $D_i^2 = (X_1 - X_2)^2/(\delta_1^2 + \delta_2^2)$  can now be put as:  $D_i^2 = (Q - 1)^2/(Q^2 q_1^2 + q_2^2)$ . Discrepancy quotient, Q, will be useful in our discussion, if we impose the condition that  $Q \geq 1$ , no matter whether the attribute in the crime sample is greater than that in the source or *vice versa*. It will indicate a Q-fold discrepancy in the value of the attribute in the crime sample and source and calculations of the discrepancy index (*vide infra*) will be to the benefit of the accused. We now define  $\Delta_i$ , a normal deviate with unit standard deviation as

$$\Delta_i^2 = (1/Q - 1)^2/(k')^2, \text{ where } k'/(1/Q) = q.$$

∵ if  $(X_2 \pm \delta_2)/(X_1 \pm \delta_1) = (1/Q \pm k')$ , we have,  $k'/(1/Q) = q$ . For identity of crime sample and source, we have  $Q = 1$ ,  $(1/Q - 1) = 0$ . From the above we get

$$\Delta_i^2 = (Q_i - 1)^2/q_i^2 \quad (2)$$

The modified discrepancy index,  $\theta$ , may now be compared with the critical value  $\theta_0$  from  $\chi^2$  tables to test the match.

$$\theta = \sum_{i=1}^N \Delta_i^2 = \sum_{i=1}^N (Q_i - 1)^2/q_i^2. \quad (3)$$

when

$$Q \rightarrow 1, \Delta_i^2 \rightarrow D_i^2.$$

It may appear that when  $X_1$  and  $X_2$  are (presumed to be) normally distributed,  $D^2$  is  $\chi^2$  distributed, then  $\Delta_i^2$  cannot be  $\chi^2$  distributed. In fact "a Gaussian distribution may be inappropriate for measurements, such as chemical composition, which must be always non-negative, a functional transform whereby the new variate is brought into closer alignment with the Gaussian distribution may be used instead. The logarithm of the chemical composition could be used rather than the chemical composition itself<sup>2(a)</sup>. Indeed the log-concentration of elements in human hair has been found to be closely Gaussian<sup>2(b)</sup>. On the first presumption  $X_2 - X_1$  is a Gaussian variate with zero mean and standard deviation  $\sqrt{\delta_1^2 + \delta_2^2}$ . Now, if on the other hand  $\ln X_1$  and  $\ln X_2$  are admittedly better Gaussian distributed, and if the sample could stem from the alleged source,

$$\ln X_2 - \ln X_1 = \ln (X_2/X_1) = \ln \left( 1 + \frac{X_2 - X_1}{X_1} \right) \approx \frac{X_2 - X_1}{X_1} = \left( \frac{1}{Q} - 1 \right)$$

is a Gaussian variate with zero mean and standard deviation  $k' = q/Q$ , and  $\Delta_i^2$  is  $\chi^2$  distributed. The approximation involved is valid at lower discrepancies where the test for matching is sought.

It will now be shown that the use of  $c$  in place of  $\ln c$  (cf. Parker) does not make any significant difference where a critical examination is necessary. For large discrepancies, the difference in the crime sample and the alleged source is obvious; an inspection of data is sufficient. The use of  $c$  units makes evaluation of data less laborious.

Using  $c$  units, we have a series of measurements :

$$c_1 = \bar{c} + x_1, \dots, c_n = \bar{c} + x_n, \text{ where } x\text{'s can be}$$

+ve -ve, yielding the result:  $\bar{c} \pm \delta_c$  where  $\delta_c$  is the standard deviation, calculated using  $c$  units directly. To convert the measurements into  $\ln c$  units, we

$$\text{have, } \ln c_1 = \ln (\bar{c} + x_1) \approx \ln \bar{c} + x_1/\bar{c}, \text{ etc.,}$$

showing that  $\delta_{\ln c} \approx \delta_c/\bar{c}$  where  $\delta_{\ln c}$  is the standard deviation calculated using  $\ln c$  units. Parker's discrepancy index element,  $D_i^2$  using  $\ln c$  units, can now be readily shown  $\approx D_i^2$  using  $c$  units, at lower discrepancies.

$$D_i^2 = (\ln X_1 - \ln X_2)^2 / [(\delta_{c,1}/X_1)^2 + (\delta_{c,2}/X_2)^2]$$

can be shown, at lower discrepancies,  $\approx (X_1 - X_2)^2 / (\delta_{c,1}^2 + \delta_{c,2}^2)$ . Thus in Parker's eqn. (1) whether  $\ln c$  values or  $c$  values are taken, these reduce to the same form and magnitude at lower discrepancies, where the test is necessary.

**Alternative Method for Constructing a Discrepancy Index.**—The discrepancy quotient,  $Q$ , ( $Q \geq 1$ ) may be suitably modified to yield a minimum value,  $Q_{min}$ . Thus at a given chosen probability level

$Q_{min} = Q - t \cdot k$ , where ' $t$ '—times the standard deviation is subtracted on the basis of Student's  $t$  value for the given desired probability level and for the given degrees of freedom. Thus for a series of 10 measurements (9 degrees of freedom) for  $i$ -th attribute the probability of a chance deviation in  $Q$  not exceeding  $2.26 k$  is 95% ( $t = 2.26$ ). Having obtained  $Q_{min}$  values for each attribute, ( $Q_{min} \geq 1$ ), we have the alternative discrepancy index, which may now be denoted by  $\Gamma$ , simply given by :

$$\begin{aligned} \Gamma &= \prod_{i=1}^N Q_{min, i} = \prod_{i=1}^N (Q_i - t_i k_i) \\ &= \prod_{i=1}^N Q_i (1 - t_i \cdot q_i). \end{aligned} \quad (4)$$

$Q_{min}$  values  $\geq 1$  only are considered ; those working out to  $< 1$  are ignored, as these mean that there

is no significant difference in the values of the given attribute. Ideally,  $\Gamma_0$  for identity between the crime sample and the alleged source should be unity. Values of  $\Gamma$  in excess of unity would therefore indicate the extent of discrepancy between the two.

Application of the above criteria to typical literature data is given in Tables I to III. While  $\Gamma$  is well suited to the evaluation of data on chemical composition it will be apparent that in the evaluation of data on physical properties like density, refractive index, etc., in the event of high discrepancies (owing to very low  $q$ 's),  $\Gamma$  will not suitably reflect these. The discrepancy indexes  $c$  and  $\theta$  have therefore general applicability.

## 2. ASSESSMENT OF EVIDENTIAL VALUE

Once the questioned material is shown indistinguishable from the alleged source, the next consideration arises as to what significance or evidential value this agreement has. Let  $p_i$  be the probability of finding the level of the  $i$ -th attribute in the entire population similar to that in the alleged source then the probability of finding  $N$  uncorrelated attributes (which may be measured) in levels

similar to those in the alleged source is  $P_N = \prod_{i=1}^N p_i$ . The smaller the value of  $P_N$ , the more weighty is the evidence. The value of an attribute towards identification depends on "the ratio of the measurement error to the spread of the attribute over the population and also on the value of the sample measurement when referred to the peak of the frequency distribution"<sup>2(b)</sup>. In the case of normal distributions the probability  $p_i$  of finding the level of the attribute  $i$  in the entire population similar

TABLE I

Sample 1 w.r.t. "control" (NAA of human hair)<sup>2(a)</sup>

| Attribute | $q$<br>(estimated) | $D_i^2$                                |                         | $\Delta_i^2$    | $Q = 1$ | $Q_{min} = 1$             |                           |
|-----------|--------------------|--|-------------------------|-----------------|---------|---------------------------|---------------------------|
|           |                    | Using<br><i>ln c</i> units             | Using<br><i>c</i> units |                 |         | (P = 80%)<br>$Q(1-1.38q)$ | (P = 95%)<br>$Q(1-2.26q)$ |
| Na        | 0.24               | 4.1                                    | 2.5                     | 6.2             | 1.60    | 1.07                      | †                         |
| Zn        | 0.10               | 2.6                                    | 2.9                     | 2.9             | 1.17    | 1.01                      |                           |
|           |                    | Likewise for Cl, Mn, I, Cu, Br, Au, Hg |                         |                 |         |                           |                           |
| N = 9     |                    | $\Sigma = 15.1$                        | $\Sigma = 12.4$         | $\Sigma = 22.8$ |         | $H = 1.22$                | $H = 1.0$                 |

† a dash denotes  $Q_{min} = 1.00$ .

TABLE II

Summary of analysis of data on samples Nos. 1, 4, 15, 30 w.r.t. "control" (NAA of human hair)<sup>2(b)</sup>

| Sample No. | No. of attributes measured | $\Sigma D_i^2$<br>using <i>ln c</i> units | $\Sigma \Delta_i^2$ | $H Q_i(1-2.26q_i)$ | Remarks                                   |
|------------|----------------------------|---|---------------------|--------------------|---|
| 1          | 9                          | 15.1 (12.4)†                              | 22.8 (12.3)*        | 1.0                | Source similar to control                 |
| 4          | 10                         | 42.9 (33.9)†                              | 111.0 (34.0)*       | 1.28               | Source different, significant discrepancy |
| 15         | 10                         | 269.2                                     | 2760                | 17.2               | Source different, large discrepancy       |
| 30         | 9                          | $2.61_3 \cdot 10^3$                       | $2.30_6 \cdot 10^5$ | 108.3              | Source different, v. large discrepancy    |

† recalculated values, using *c* units, instead of *ln c* units.

$\Sigma_0 = 27.8$ ,  $\Sigma_0 = 29.6$  (at 0.1% probability level for  $C = C_0$  or  $\theta = \theta_0$ )

\* Calcd. without imposing the condition  $Q = 1$ ;  $Q = (X_1/X_2)^2 \geq 1$ .

TABLE III

Soils (F and K-1)—Evaluation of spectrographic (Seidel function) data<sup>5</sup>

| Attribute                                   | $q_1$ | $q_2$ | $q$  | $D_i^2$          | $\Delta_i^2$     | $Q = 1$ | $Q_{min} = 1$<br>$Q(1-2.26q)$ |
|---|-------|-------|------|------------------|------------------|---------|-------------------------------|
| Mn  | 0.20  | 0.21  | 0.29 | 11.6             | 92.9             | 3.79    | 1.31                          |
| Likewise for Al, Si, Cr, Na, Fe, Mg, Cu, Ca |       |       |      |                  |                  |         |                               |
| Ti  | 0.25  | 0.21  | 0.32 | 15.5             | 259.5            | 6.26    | 1.63                          |
| N = 10                                      |       |       |      | $\Sigma = 108.1$ | $\Sigma = 629.5$ |         | $H = 4.10$                    |

Conclusion: Soils from different sources.

to that in the crime material or the alleged source, for which the intrinsic variability of the material and the measurement errors are determined would simply be given by the ratio of areas under the respective distribution curves,  $a/A$ , where  $a$  is that under the source curve and  $A$  is that under the population curve. If the latter is plotted as  $\phi_x$  vs

$\sigma$  curve (Fig. 1) such that the normal distribution is valid:  $\phi_x = (1/\sqrt{2\pi}) \exp(-x^2/2)$  where  $x$  is measured in standard deviation  $\sigma$  units, we have, by definition, area under population curve (PQR, Fig. 1),  $A = 1$ . Therefore  $p_i = a$ , the area under the distribution curve for the source (say, ABC or DEF) which is properly constructed within the

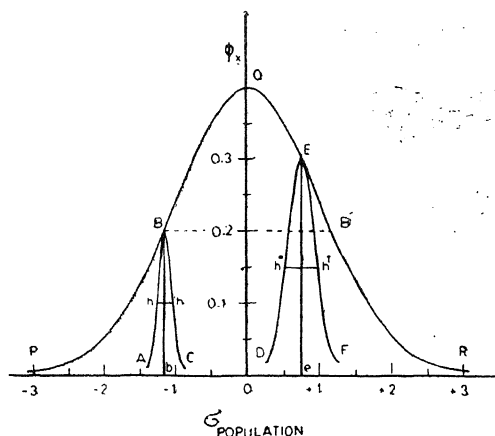


FIG. 1. Construction of a typical normal distribution curve for the alleged source material (ABC or DEF) within that of the population (PQR).  $\phi_x$ ,  $t$  in  $\sigma_{\text{population}}$  units,  $\sigma_{\text{source}}/\sigma_{\text{population}} = 0.047$ ,  $-1.17, 0.1$  and  $0.14, +0.75, 0.2$  for curves ABC and DEF respectively.

The approximation in the above equation may be corrected for by utilizing :

$$p_i = 2.51 \phi_{x,i}^* (\sigma_{\text{source}}/\sigma_{\text{population}})_i \quad (5)$$

with benefit to the accused.  $\phi_{x,0}$  ( $\phi$  corresponding to  $\sigma = 0$ ) = 0.3989, is equivalent to area.  $A = 1$  (vide supra). Therefore, area equivalent to  $\phi_{x,i}^* = (1/0.3989) \phi_{x,i}^* (\sigma_{\text{source}}/\sigma_{\text{population}})$ , the same as in eqn. (5).

*Illustration.*—The calculation of  $P_N$  for a sample human hair<sup>2(b)</sup> is illustrated in Table IV. The analysis utilizing eqn. (5) would thus reveal, granted that our assumption of (log —) normal distribution over the population is valid and that the attributes are uncorrelated, that there is 1 in  $\sim 10^7$  chance of finding such hair in the population in question, with levels of the ten attributes in question similar to those in the given sample. The magnitude of  $P_N$  is indicative of the uniqueness of the given physical evidence material. It is an attempt to quantitate the evidential value.

TABLE IV

Uniqueness of crime material—Human hair in "control" sample<sup>2(b)</sup>

| Attribute | $\frac{\mu_{\text{control}} - \mu_{\text{population}}}{\sigma_{\text{population}} = t}$ | $\phi_{x,i}^*$<br>(corresponding to $t$ ) | $p_i = 2.36 \phi_{x,i}^* \left( \frac{\sigma_{\text{control}}}{\sigma_{\text{population}}} \right)$ |
|-----------|---|---|---|
| Na        | 0.442   | 0.362                                     | 0.201   |
| Sb        | 0.855   | 0.277                                     | 0.284   |
|           | Likewise for Cl, Mn, I, Cu, Br, Au, Hg, Zn  |   |   |

$$P_N = 1.1 \times 10^{-7} \text{ (Eqn. 5)}$$

$$P_N = \prod p_i = 0.58 \times 10^{-7}$$

population curve. Evaluate  $t = |\mu_{\text{population}} - \mu_{\text{source}}| / \sigma_{\text{population}}$ , where  $\mu$  is the mean value. The crime material or the source is therefore  $t$  units of population-standard deviations removed from the population mean value. Construct a normal curve on the ordinate at  $t$  converting  $\sigma_{\text{source}}$  into  $\sigma_{\text{population}}$  units, by the factor  $(\sigma_{\text{source}}/\sigma_{\text{population}})$ . The area under the source curve,  $a$ , can be evaluated as  $a \approx \phi_{x,i}^*$  (H.I.B.W.) where  $\phi_{x,i}^*$  is the ordinate corresponding to the  $t$  value (e.g., Bb or Ee) which can be directly read from the normal error curve tables. The half-intensity band width, H.I.B.W., (e.g.,  $hh'$  or  $h'h'$ ) =  $2.36 (\sigma_{\text{source}}/\sigma_{\text{population}})$  for the source curves. H.I.B.W. =  $2.36 \sigma$  units for any normal curve. We have, therefore,

$$p_i = a_i = 2.36 \phi_{x,i}^* (\sigma_{\text{source}}/\sigma_{\text{population}})_i$$

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## XVII INTERNATIONAL CONGRESS OF MATHEMATICIANS

**T**HE International Congress of Mathematicians is held once every four years. The XVII Congress met at the University of British Columbia, Vancouver, Canada, from August 21–29, 1974. The last Congress was held in Nice France in 1970. The Congress was attended by about 3000 mathematicians from all over the world. A few of its sessions were also held at the Universities of Victoria and Simon Fraser.

The Conference was inaugurated on August 21 by Lieut. Governor of British Columbia followed by addresses of the President and the Mayor of Vancouver at the Queen Elizabeth Theatre. This year only two gold medals were awarded—one to Enrico Bombieri of Pisa University, Italy, for number theory, and the other to David B. Mumford of Harvard University for Algebraic Geometry.

During the Conference seventeen expository addresses, 160 short communications and 750 Research papers were presented. About 50 Indians of whom 40 were already residents abroad presented their papers at the Congress. They included C. R. Rao, V. K. Patodi, V. S. Varadarajan, B. R. Seth, R. S. Mishra, H. R. Gupta, M. K. Singhal, P. Puri, M. N. L. Narasimhan, P. N. Kaloni, B. D. Agrawal, S. M. Sah, V. M. Soundalgekar.

The general lectures dealt with potential theory, Fourier analysis, partial differential equations, eigenvalues of the Laplacian and invariants of manifolds, tidal energy, quantum field theory, transversal theory, mathematical theory of economic equilibrium, theory of buildings, number theory and uniform approximation by holomorphic functions. The general lectures were given in the morning and the presentation of papers was done in a number of sections in the afternoon. Those dealing with mathematical physics and mechanics continued for five days. There was good interest shown in bi-mathematics, history and education. There were a number of sessions on topology, group theory, complex and real analysis, partial differential equations, logic and foundations and functional analysis.

The resurgence of the study of geometrical bodies inside a manifold was discussed by Dennis Sullivan. The qualitative study of dynamical systems produces inside one manifold interesting compact subsets, families of intertwined non-compact submanifolds, geometrically defined measures and currents, with homological interpretations and relationships. A. G. Vitushkin showed that recent advances in uniform approximations of holomorphic functions mainly consist in the improvement of integral representa-

tions, estimation of the solutions and the construction of concrete examples to correct hypotheses.

G. F. D. Duff dealt with the partial differential equations of the tidal motions which form a symmetric hyperbolic system in two space dimensions with a monotone non-linearity in a variable domain. C. Radhakrishna Rao gave a general survey of recent results on characterization problems and their applications to testing of hypothesis, estimation of parameters, inference on unobservable structural variables and specification problems. An account of Harish Chandra's work on harmonic analysis on real semisimple Lie group with special emphasis on the Plancherel formula was given by V. S. Varadarajan. He also discussed matrix coefficients and the theory of eigenfunction expansions, infinitesimal theory of representations and intertwining operators for irreducible representations. Masahisa Inoue gave new examples of surfaces with affine structures.

B. R. Seth showed that the transition region may be interpreted as : (i) an asymptotic subspace ; or (ii) a criticality or singularity of the differential manifold defining the medium ; or (iii) a change of group symmetry ; or (iv) a singular transformation matrix. It is found that transition fields are sub or super-harmonic and are characterised by spin, rotation or vorticity effects. M. N. L. Narasimhan and A. C. Eringen gave balance laws governing the flow of heat-conducting nematic liquid crystals. A generalized form of the Clausius-Duhem inequality gives the effects of heat conduction. Gilbert Strang proved that in the non-linear applications of the Finite Element Method the first step improves the error involved from  $o(h)$  to  $O(h^{3/2})$ ,  $h$  being the mesh-width.

R. Arthur Knebel generalised Kleene's theorem on automata to universal algebra. R. Padmanabhan proved that any uniquely complemented lattice belonging to the class K is distributive.

Michael Doob showed that several classes of graphs are magic.

Hansraj Gupta dealt with a number of Magic partitions.

V. Krishnamurthy showed that the counting of  $T_0$ -topologies may be reduced to the counting of a special type of bichromatic trees.

Wolfgang Weil communicated the result that an arbitrary convex body in three dimensions of constant brightness and of constant width must be a ball.

B. R. SETH.



## OBITUARY

PROFESSOR LAKSHMISWARA RAMA RAO, M.A., F.G.S., F.N.A., F.A.SC.

(1896-1974)

By

PROF. S. SAMBE GOWDA

*Department of Geology, Central College, Bangalore*

**I**N the death of Prof. Lakshmiswara Rama Rao on November 11, 1974 at the age of seventy-eight the country has lost a great *acharya*, a renowned geologist, and an able administrator of cardinal virtues.

After declining the offer by the Government to join the Civil Service soon after coming out successfully with the 'CROMARTY PRIZE' at the degree examination of the Madras University in 1917, he took to his teaching career at the Central College and never regretted for the same. He perfected the art of teaching with his inborn talent and no superlatives will suffice to describe him as a teacher. Prof. Rao took to his research in right earnest at the very beginning of his career on the Upper Cretaceous rocks found along the east coast in South India and continued till to the last minute of his life, for over half a century! The discovery of identifiable remains of Dinosaurs, the discovery of microfossils such as Radiolarians in the rocks of the Utatur division, the algal genus *Archaeolithothamnium* in the Niniyur rocks, the identification of a larger foraminifer in the Pondicherry rocks as *Discocycliella* formed the main lines of his early investigations.

In his Presidential address to the Geology section of the Indian Science Congress Association in 1940, he reviewed the investigations on "Cretaceous-Tertiary Boundary" and the topic became a subject of investigation in all the continents by leading geologists. He presided over the section on Cretaceous-Tertiary Boundary at the 22nd International Geological Congress in New Delhi, and his review appeared in a special publication of the Mysore Geologists' Association. He was invited in 1972 on behalf of the International Union

of Geological Sciences by the Soviet Academy of Sciences to take part as a member of the Commission on Cretaceous-Tertiary Boundary, a fitting tribute to his sustained and serious pursuit of his chosen and choicest investigation! This coincided with the award of a medal by the Asiatic Society of Bengal in the same year.

Professor Rama Rao laid the foundation for micropalaeontological research in India. Thanks to his vision, the country has come of age in this field. It was a great day for him when the First Indian Colloquium on Micropalaeontology was inaugurated by him in Bangalore in 1971. His ambition was to organise an association of micropalaeontologists and it would be a fitting memorial to him if this Association is established on a permanent basis.

Prof. Rao played a leading and vital role in the founding of almost all the prestigious and national scientific associations, academies and the institutes including the *Current Science Association*. A special mention, however, must be made here of the great effort he made in founding the Geological Society of India and in steering the same to its present status and in bringing credit to its journal which he edited till he breathed his last.

As the Principal of Central College and as the Head of the Department of Geology, Central College with a long innings Prof. Rao has left his footprints of perfection, purity, unbiassed approach, great integrity and unimpeachable acts.

May his soul rest in peace and may his spirit inspire all those who want to work for the cause of Geology.

## LETTERS TO THE EDITOR

### DISSOCIATION ENERGY OF ALKALI HALIDE MOLECULES\*

#### ABSTRACT

Expressions for the dissociation energy have been obtained by consideration of three logarithmic potential energy functions suggested recently. The values of the dissociation energies of twenty diatomic alkali halide molecules have been calculated with the help of these expressions. The results obtained are encouraging.

**Key Words.**—Electron affinity, Ionisation potential, Binding energy, Force Constant.

In actual practice none of the ionic molecules is transformed to the separate ions under any observable conditions since ionisation energy always exceeds the electron affinity. The lowest ionisation energy, that of cesium  $377 \text{ kJ mol}^{-1}$ , is higher than the highest electron affinity,  $356 \text{ kJ/mole}$ , that of chlorine. For most compounds the difference is much greater. The gaseous atoms are, therefore, at room temperature, always more stable than the gaseous ions. Thus the binding energy represents only the hypothetical values for the unrealizable processes, whereas dissociation energy represents the more realistic values. The dissociation energy of ionic molecules is of much interest. Up to the present time only limited attempts have been made<sup>1</sup> to calculate dissociation energy using the interaction potential energy functions. The potential functions used previously have been discredited by Thakur<sup>2</sup> and Dobbs *et al.*<sup>3</sup>. Thakur<sup>2,4,5</sup> has suggested three logarithmic forms of the interaction potential functions which have been widely used<sup>6</sup> to calculate several properties of the ionic salts. In the light of these facts and the fact that the values of  $r_e$ ,  $E$  and  $k_e$  used by Tandon<sup>1</sup> were not so accurate it is worthwhile to recalculate the dissociation energies. The present note is concerned with the theoretical computation of the dissociation energies of alkali metal halides using more realistic interaction potentials and molecular constants known to a higher degree of accuracy.

The new potential energy functions are :

$$U(r) = -\frac{e^2}{r} + C \log \left[ 1 + \frac{p}{r^4} \right] \quad (1)$$

$$U(r) = -\frac{e^2}{r} + B \log \left[ 2 + \frac{b}{r^2} \right] \quad (2)$$

$$U(r) = -\frac{e^2}{r} + D \log \left[ 4 + \frac{d}{r} \right] \quad (3)$$

where  $C$ ,  $p$ ,  $B$ ,  $b$ ,  $D$  and  $d$  are potential parameters and the rest terms have their usual meanings. The

potential parameters may be calculated using molecule stability and force constant conditions in the conventional<sup>2,4,5</sup> way. Thus binding energy  $D_i$  is given by

$$D_i = -U(r_e) \quad (4)$$

and dissociation energy  $D_e$  is given<sup>2,4,5</sup> by

$$D_e = E - I + D_i \quad (5)$$

where  $E$  is the electron affinity of halogen atoms and  $I$  is the ionisation energy of alkali metals.

The application of these conditions to potential functions (1)–(3) yields :

$$D_e = E - I + \frac{e^2}{r_e} \left[ 1 - \left( \frac{r_e^4 + p}{4p} \right) \times \log \left( 1 + \frac{p}{r_e^4} \right) \right] \quad (6)$$

$$D_e = E - I + \frac{e^2}{r_e} \left[ 1 - \left( \frac{2r_e^2 + b}{2b} \right) \times \log \left( 2 + \frac{b}{r_e^2} \right) \right] \quad (7)$$

$$D_e = E - I + \frac{e^2}{r_e} \left[ 1 - \left( \frac{4r_e + d}{d} \right) \times \log \left( 4 + \frac{d}{r_e} \right) \right] \quad (8)$$

The values of the molecular parameters used in calculation have been taken from various sources of the literature<sup>7–11</sup> and are listed in Table I.

TABLE I  
Molecular constants used in calculation

| Molecule | $r_e$<br>(Å) | $k_e$<br>( $10^5/\text{cm}$ ) | $E$<br>(kJ/mole) | $I$<br>(kJ/mole) |
|----------|--------------|-------------------------------|------------------|------------------|
| LiF      | 1.545        | 2.4586                        | 327.06           | 520.0            |
| NaF      | 2.000        | 1.465                         | 327.06           | 495.8            |
| KF       | 2.171        | 1.2038                        | 327.06           | 418.7            |
| RbF      | 2.265        | 1.3913                        | 327.06           | 402.9            |
| CsF      | 2.345        | 1.4500                        | 327.06           | 375.5            |
| LiCl     | 2.037        | 1.4982                        | 353.13           | 520.0            |
| NaCl     | 2.361        | 1.1004                        | 353.13           | 495.8            |
| KCl      | 2.667        | 0.8640                        | 353.13           | 418.7            |
| RbCl     | 2.787        | 0.7666                        | 353.13           | 402.0            |
| CsCl     | 2.906        | 0.7195                        | 353.13           | 375.5            |
| LiBr     | 2.1704       | 1.2467                        | 295.22           | 520.0            |
| NaBr     | 2.502        | 0.9582                        | 295.22           | 495.8            |
| KBr      | 2.821        | 0.7009                        | 295.22           | 418.7            |
| RbBr     | 2.945        | 0.6697                        | 295.22           | 402.9            |
| CsBr     | 3.072        | 0.5675                        | 295.22           | 375.5            |
| LiI      | 2.392        | 0.9719                        | 309.70           | 520.0            |
| NaI      | 2.711        | 0.7626                        | 309.70           | 495.8            |
| KI       | 3.048        | 0.5264                        | 309.70           | 418.7            |
| RbI      | 3.177        | 0.4924                        | 309.70           | 402.9            |
| CsI      | 3.315        | 0.3897                        | 309.70           | 375.5            |

TABLE II

Calculated values of dissociation energies  
of alkali halide molecules

(in kJ/mole)

| Molecules | De<br>(calc)<br>Pot (1) | De<br>(calc)<br>Pot (2) | De<br>(calc)<br>Pot (3) | De<br>(exptl)<br>Gaydon |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|
| LiF       | 603.1                   | 602.1                   | 630.0                   | 574.1 ± 19.3            |
| NaF       | 458.4                   | 452.6                   | 457.4                   | 448.6 19.3              |
| KF        | 488.2                   | 492.1                   | 493.0                   | 482.4 24.1              |
| RbF       | 513.7                   | 489.5                   | 474.1                   | 516.2 19.3              |
| CsF       | 505.4                   | 488.0                   | 482.2                   | 530.6 19.3              |
| LiCl      | 480.8                   | 448.1                   | 455.4                   | 482.4 28.9              |
| NaCl      | 402.4                   | 385.1                   | 389.9                   | 409.1 4.8               |
| KCl       | 418.8                   | 403.3                   | 413.0                   | 424.4 4.8               |
| RbCl      | 412.4                   | 399.8                   | 408.5                   | 434.2 19.3              |
| CsCl      | 423.4                   | 409.9                   | 417.6                   | 443.8 19.3              |
| LiBr      | 364.7                   | 346.4                   | 378.2                   | 419.7 28.9              |
| NaBr      | 314.6                   | 300.1                   | 310.8                   | 366.6 9.6               |
| KBr       | 334.8                   | 319.4                   | 336.7                   | 380.1 4.8               |
| RbBr      | 337.1                   | 317.8                   | 336.1                   | 385.9 24.1              |
| CsBr      | 334.2                   | 326.9                   | 337.5                   | 395.6 24.1              |
| LiI       | 329.0                   | 309.7                   | 336.7                   | 337.7 19.3              |
| NaI       | 289.6                   | 274.1                   | 277.0                   | 296.2 9.6               |
| KI        | 314.6                   | 300.1                   | 319.4                   | 320.3 4.8               |
| RbI       | 315.9                   | 300.5                   | 323.6                   | 323.2 9.6               |
| CsI       | 313.4                   | 288.3                   | 322.1                   | 328.0 9.6               |

Table II presents the calculated values of  $D_e$  from Equations (6)–(8). The experimental values of  $D_e$  in Table II are of Gaydon<sup>12</sup>. The agreement between the theoretical and experimental values is fair. Thus we find that the new potential functions, which give good values of several constants, yield good values of dissociation energy also.

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### X-RAY, DIELECTRIC AND RESISTIVITY STUDIES OF BaGeO<sub>3</sub>

THE  $A^{2+}B^{4+}O_3$  compounds with  $Ge^{4+}$  as B ion seem to have attracted very little attention though some of these have been found to exhibit interesting properties<sup>1,2</sup>. Here we report the structural and electrical properties of BaGeO<sub>3</sub>.

*Preparation of the compound.*—The two oxides BaO–GeO<sub>2</sub> (purity above 99.99%) are mixed in 1:1 molar proportion under acetone. The mixture was then dried in air. It was heated in a platinum crucible for about 50 hours at 750° C. The mass was then slowly cooled to room temperature. BaGeO<sub>3</sub> is found to be a pinkish coloured powder. The compound is not hygroscopic.

*X-Ray study.*—The structure of the compound was investigated using 114.6 mm diameter Debye-Scherrer camera and filtered CuK<sub>α</sub> radiation. A comparison of the  $d$  values and the structure of the compound synthesised by us with those reported earlier<sup>3</sup> show a clear disagreement. BaGeO<sub>3</sub> is found to have an orthorhombic cell with  $a = 6.09$  Å,  $b = 4.85$  Å, and  $c = 6.52$  Å. The observed and calculated  $d$  values are given in Table I. The

TABLE I  
X-ray diffraction data for BaGeO<sub>3</sub>

| (hkl) | $d$<br>Observed | $d$<br>Calculated | Intensity<br>observed |
|-------|-----------------|-------------------|-----------------------|
| (010) | 4.849           | 4.876             | V.W.                  |
| (110) | 3.849           | 3.808             | V.W.                  |
| (002) | 3.247           | 3.258             | M.                    |
| (200) | 3.033           | 3.049             | M.                    |
| (102) | 2.906           | 2.873             | S.                    |
| (012) | 2.695           | 2.708             | M.                    |
| (112) | 2.512           | 2.475             | S.                    |
| (022) | 1.974           | 1.952             | V.W.                  |
| (310) | 1.868           | 1.875             | V.W.                  |
| (311) | 1.815           | 1.802             | V.W.                  |
| (203) | 1.744           | 1.769             | W.                    |
| (302) | 1.695           | 1.724             | W.                    |
| (030) | 1.646           | 1.626             | M.                    |
| (031) | 1.595           | 1.577             | M.                    |
| (400) | 1.522           | 1.524             | W.                    |
| (401) | 1.482           | 1.484             | V.W.                  |
| (230) | 1.440           | 1.434             | V.W.                  |
| (402) | 1.369           | 1.380             | V.W.                  |
| (024) | 1.351           | 1.354             | V.W.                  |

W: Weak, M: Medium, S: Strong, V.W.: Very weak.

absence of GeO<sub>2</sub> lines as well as the presence of new unit cell indicates the formation of the compound. The difference between the structure of BaGeO<sub>3</sub> reported earlier and that reported here is

probably due to the prolonged heating at a lower temperature during its preparation<sup>1</sup>.

Goldschmidt<sup>5</sup> has shown that compounds with general formula  $A^{2+}B^{++}O_3$  possess a cubic perovskite structure if a tolerance factor defined by

$$R_A + R_O = t \sqrt{2(R_B + R_O)}$$

has values between 0.77 and nearly one and a somewhat larger range for distorted perovskite structures.  $BaGeO_3$  with  $t = 1.007$  would therefore be expected to possess similar structure. Hence intensity calculations for all the observed reflections were carried out assuming orthorhombic  $BaTiO_3$  type structure (Bmm2). Very little agreement is found between the calculated and the observed intensities indicating that the structure of  $BaGeO_3$  is different from that of  $BaTiO_3$  (perovskite type).

**Resistivity and Dielectric study.**—The compound was pressed into pellets for studying its electrical properties. The finely ground powder of the compound was mixed with a little binder (PVA) and pressed into pellets of 1 cm diameter and 2 mm thickness under pressure of 10 ton/Sq inch in a hydraulic press. The pellets were then heated to 200° C for a few hours in a furnace to remove the binder. The temperature was then raised to 750° C and maintained there for six hours and then slowly lowered to room temperature. The dielectric constant at 1 KHz and  $d c$  resistivity of the pellets were measured by Marconi Universal Bridge type TF 868/1 and B.P.L. meg-meg-ohm-meter type RM 160/3 respectively. The variation of dielectric constant with temperature is shown in Fig. 1 and that of resistivity in Fig. 2. The resistivity of  $BaGeO_3$

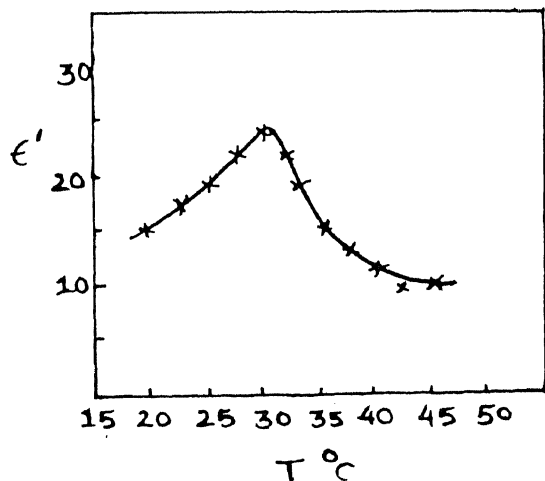


FIG. 1. Variation of  $\epsilon'$  with temperature.

shows a marked anomaly at 32° C but the dielectric constant shows an anomaly at a slightly lower

temperature. The anomalous behaviour is observed in all the pellets on heating but not reproduced in the cooling cycles. Identical results were obtained on repeating the measurements a number of times. The response to heating is spontaneous. This type of behaviour is also reported in  $RbNO_3$ <sup>6</sup>. The detailed investigations of the nature of anomaly, space group, and high temperature phase are in progress.

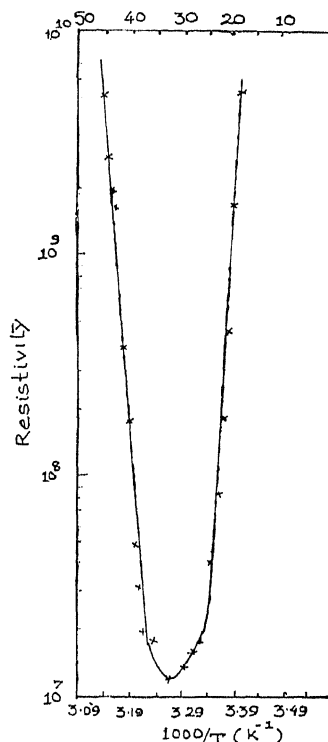


FIG. 2. Variation of resistivity with  $1000/T$  ( $K^{-1}$ ).

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**SYNTHESIS OF SOME NEW ARYL- AND  
ARYLOXYALKYL-N-(5-NITRO-2-FURYL)-  
CARBAMATES AS POTENTIAL  
ANTIMICROBIAL AGENTS**

5-NITROFURAN derivatives are well known for their antibacterial<sup>1</sup> activity. Also many carbamates have been found to possess diverse types of biological activity including antifungal<sup>2</sup> and anthelmintic<sup>3</sup> activities, etc. No biological activity is described for alkyl-N-(5-nitro-2-furyl) carbamates known in literature<sup>4,5</sup>. Furthermore, the presence of aryloxy groups in many antimicrobial agents<sup>6</sup> seems to be their important feature. Fifteen new aryl- and aryloxyalkyl-N-(5-nitro-2-furyl) carbamates (I and II) have been synthesised with a view to evaluate their antifungal and antibacterial activities.

5-Nitro-2-furyl azide was made following the method of Singleton and Edwards<sup>7</sup> by treating an ethereal solution of 5-Nitro-2-furoyl chloride with aqueous sodium azide at 0–5° C when it separated out as an yellow crystalline solid. I.R. (Nujol, cm<sup>-1</sup>): 2140 s (N=N=N) and 1680 s (C=O). The final carbamates were obtained by condensing various phenols and aryloxyalkanols with 5-nitrofuryl isocyanate obtained *in situ* from above 5-nitrofuroyl azide.

In a typical experiment for the preparation of the aryl carbamates, 5-nitro-2-furoyl azide (0.01 moles) in dry benzene (25 ml) was heated for 4 hours at 75° to liberate 5-nitro-2-furyl isocyanate (small quantity of black powder separated out due to

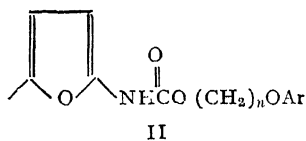
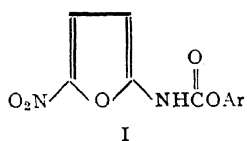
polymerisation). Phenol (0.011 moles) in dry benzene (25 ml) was added to 5-nitro-2-furyl isocyanate, thus obtained, and heating was further continued for 6 hours. Ether (100 ml) was added to the cooled reaction mixture, organic layer washed with 10% hydrochloric acid followed by water and dried (Na<sub>2</sub>SO<sub>4</sub>). Removal of the organic solvent gave brownish yellow solid which was taken in benzene and chromatographed over silica gel. Elution with benzene afforded phenyl-N-(5-nitro-2-furyl) carbamate as yellow shining needles, which was further purified by recrystallising from benzene-petroleum ether (40–60° C).

The aryloxyalkyl carbamates were prepared by heating the azide and aryloxyalkanols directly without a solvent at 70° till the evolution of nitrogen ceased. The products were worked up as usual.

All the carbamates showed characteristic I.R. absorptions (Nujol, cm<sup>-1</sup>): 3230 m (NH) and 1720 s (ester). Table I gives the details of various compounds prepared together with their melting points, elemental analyses and percentage yields. All compounds were recrystallised from benzene-petroleum ether (40–60°).

All the fifteen carbamates were tested *in vitro* for antifungal activity by agar dilution assay method<sup>8</sup> and for antibacterial activity by serial dilution tube method<sup>9</sup> using various pathogenic test organisms. Some of the compounds were found to possess moderate activity as shown in Table II.

TABLE I



| Compound No. | Structure | n  | Ar                      | m.p. °C    | Yield % | Molecular formula   | % C   |        | % H   |        |
|--------------|-----------|----|-------------------------|------------|---------|---|-------|--------|-------|--------|
|              |           |    |                         |            |         |   | Found | Calcd. | Found | Calcd. |
| 1            | I         | .. | Phenyl                  | 108–10     | 50      | C <sub>11</sub> H <sub>8</sub> N <sub>2</sub> O <sub>5</sub>    | 53.38 | 53.22  | 3.65  | 3.22   |
| 2            | I         | .. | p-Chlorophenyl          | 176–78     | 70      | C <sub>11</sub> H <sub>7</sub> ClN <sub>2</sub> O <sub>5</sub>  | 46.65 | 46.73  | 2.70  | 2.47   |
| 3            | I         | .. | p-Tolyl                 | 121–23     | 66      | C <sub>12</sub> H <sub>10</sub> N <sub>2</sub> O <sub>5</sub>   | 55.44 | 54.96  | 4.00  | 3.81   |
| 4            | I         | .. | Thymyl                  | 124–26     | 50      | C <sub>15</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>   | 59.27 | 59.21  | 5.35  | 5.26   |
| 5            | I         | .. | 4-Chlorothymyl          | 138–40     | 54      | C <sub>15</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>5</sub> | 53.56 | 53.19  | 4.59  | 4.43   |
| 6            | I         | .. | o-Allyl-p-chloro-phenyl | 127–29 (d) | 75      | C <sub>14</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>5</sub> | 52.20 | 52.08  | 3.32  | 3.41   |
| 7            | II        | 2  | Phenyl                  | 104–5      | 48      | C <sub>13</sub> H <sub>12</sub> N <sub>2</sub> O <sub>6</sub>   | 53.90 | 53.42  | 4.55  | 4.11   |
| 8            | II        | 2  | p-Chlorophenyl          | 126–28     | 52      | C <sub>13</sub> H <sub>11</sub> ClN <sub>2</sub> O <sub>6</sub> | 48.09 | 47.77  | 3.40  | 3.37   |
| 9            | II        | 2  | 3-Nitrophenyl           | 162–64 (d) | 65      | C <sub>13</sub> H <sub>11</sub> N <sub>3</sub> O <sub>8</sub>   | 46.50 | 46.29  | 3.54  | 3.26   |
| 10           | II        | 2  | Thymyl                  | 98–100     | 60      | C <sub>17</sub> H <sub>20</sub> N <sub>2</sub> O <sub>6</sub>   | 58.43 | 58.61  | 5.81  | 5.74   |
| 11           | II        | 2  | 4-Chlorothymyl          | 90–92      | 65      | C <sub>17</sub> H <sub>19</sub> ClN <sub>2</sub> O <sub>6</sub> | 52.89 | 53.32  | 4.41  | 4.96   |
| 12           | II        | 4  | Phenyl                  | 108–10     | 62      | C <sub>15</sub> H <sub>16</sub> N <sub>2</sub> O <sub>6</sub>   | 56.51 | 56.26  | 5.30  | 5.00   |
| 13           | II        | 4  | p-Chlorophenyl          | 152–53     | 46      | C <sub>15</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>6</sub> | 50.93 | 50.78  | 4.54  | 4.23   |
| 14           | II        | 6  | p-Chlorophenyl          | 130–32     | 57      | C <sub>17</sub> H <sub>19</sub> ClN <sub>2</sub> O <sub>6</sub> | 53.36 | 53.32  | 4.66  | 4.96   |
| 15           | II        | 6  | Thymyl                  | 102–4      | 75      | C <sub>21</sub> H <sub>28</sub> N <sub>2</sub> O <sub>6</sub>   | 62.24 | 62.37  | 6.82  | 6.93   |

TABLE II  
Antifungal and antibacterial activities M. I. C. ( $\mu$  g/ml)

| (a)      |     | Fungi |      |      |      |      |      |      |      |      |      |
|----------|-----|-------|------|------|------|------|------|------|------|------|------|
| Compound | No. | T.m.  | T.r. | M.c. | M.g. | C.a. | C.n. | S.s. | H.c. | A.f. | A.t. |
|          | 1   | ..    | ..   | ..   | 100  | ..   | ..   | 100  | 25   | ..   | ..   |
|          | 2   | 100   | ..   | ..   | ..   | ..   | ..   | 100  | 100  | ..   | ..   |
|          | 3   | 100   | 100  | ..   | 100  | ..   | ..   | 100  | 100  | ..   | 100  |
|          | 4   | 50    | ..   | 100  | 100  | ..   | ..   | 100  | 25   | ..   | ..   |
|          | 5   | 50    | 50   | 25   | 25   | ..   | ..   | 50   | 25   | ..   | ..   |
|          | 6   | 50    | 50   | 50   | 50   | 100  | 100  | 50   | 25   | ..   | 100  |

| (b) |    | Bacteria |      |      |      |       |      |       |  |
|-----|----|----------|------|------|------|-------|------|-------|--|
|     |    | S.a.     | S.f. | E.c. | K.p. | Ps.a. | S.t. | Ag.t. |  |
|     | 5  | 50       | 25   | ..   | 50   | 25    | ..   | 50    |  |
|     | 6  | ..       | ..   | ..   | ..   | 100   | ..   | ..    |  |
|     | 11 | ..       | ..   | ..   | 100  | 25    | ..   | 25    |  |
|     | 13 | ..       | ..   | ..   | ..   | ..    | ..   | 50    |  |

Fungi: T.m.=*Trichophyton mentagrophytes*; T.r.=*Trichophyton rubrum*; M.c.=*Microsporum canis*; M.g.=*Microsporum gypseum*; C.a.=*Candida albicans*; C.n.=*Cryptococcus neoformans*; S.s.=*Sporotrichum schenckii*; H.c.=*Histoplasma capsulatum*; A.f.=*Aspergillus fumigatus*; A.t.=*Alternaria tenuis*.

Bacteria: S.a.=*Staphylococcus aureus*; S.f.=*Streptococcus faecalis*; E.c.=*Escherichia coli*; K.p.=*Klebsiella pneumoniae*; Ps.a.=*Pseudomonas aeruginosa*; S.t.=*Salmonella typhi*; Ag.t.=*Agrobacterium tumefaciens*; ..=Inactive

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### INFLUENCE OF SOLVENTS ON THE CHELATION IN BENZOIN AND METHYL MANDELATE

INTRAMOLECULAR hydrogen bonding in six membered chelates as formed in salicylaldehyde, methyl salicylate, 2-hydroxy acetophenone and enols of  $\beta$ -diketones has been studied earlier<sup>1</sup>. The intramolecular hydrogen bonding leading to the formation of five membered chelate, however, received little attention.

In this communication, evidence for chelation in benzoïn and methyl mandelate is furnished. With a view to studying the influence of solvents on chelation the pmr spectra of these compounds have been recorded on Varian A. 60 D in  $\text{CS}_2$ ,  $\text{CDCl}_3$ , DMSO, DMF, acetone and in the presence of traces of trifluoroacetic acid and methanol using TMS as internal standard at 37°C. The

concentrations of the solutions used are of the order of 0.1 molar. The results are presented in Table I.

TABLE I

*CH and OH chemical shifts of benzoin and methyl mandelate in different solvents*

| Solvent                | Benzoin              |                      | Methyl mandelate     |                      |
|------------------------|----------------------|----------------------|----------------------|----------------------|
|                        | CH<br>$\delta$ (ppm) | OH<br>$\delta$ (ppm) | CH<br>$\delta$ (ppm) | OH<br>$\delta$ (ppm) |
| CS <sub>2</sub>        | 5.73                 | 4.10                 | 4.90                 | 3.22                 |
|                        | 5.83                 | 4.20                 | 5.00                 | 3.32                 |
| CDCl <sub>3</sub>      | 5.90                 | 4.52                 | 5.20                 | 3.30                 |
|                        | 6.00                 | 4.62                 | 5.30                 | 3.40                 |
| „ + D <sub>2</sub> O   | 5.90                 | ..                   | 5.20                 | ..                   |
| „ + TFA                | 6.10                 | ..                   | 5.58                 | ..                   |
| „ + CH <sub>3</sub> OH | 6.00                 | ..                   | 5.17                 | ..                   |
| Acetone                | 6.10                 | 4.85                 | 5.20                 | 4.67                 |
|                        | 6.20                 | 4.95                 | 5.30                 | 4.77                 |
| DMF                    | 6.15                 | 5.85                 | 5.23                 | 5.87                 |
|                        | 6.25                 | 5.95                 | 5.36                 | 5.97                 |
| DMSO                   | 5.90                 | 6.05                 | 5.13                 | 5.95                 |
|                        | 6.00                 | 6.15                 | 5.22                 | 6.05                 |

TFA = Trifluoro acetic acid

DMF = Dimethylformamide

DMSO = Dimethylsulphoxide

The spectra of benzoin and methyl mandelate were reported earlier<sup>2,3</sup> but no study has been made in regard to chelation in these compounds. Both the compounds are found to exhibit doublets ( $J = 6.0$  Hz) for proton signals of OH and CH of CHOH group. This is in contrast to the earlier report<sup>2</sup> where singlets were reported for OH and CH protons. The splitting of OH and CH signals may be due to coupling between them. In presence of D<sub>2</sub>O, trifluoroacetic acid and methanol, the OH signals vanished and the CH signals collapsed into singlets. Such a collapse of the fine structure of the proton signals due to chemical exchange was reported earlier in the case of methanol<sup>4</sup>. In the presence of acids or bases the CH<sub>3</sub> doublet of methanol collapses into a singlet due to rapid OH proton exchange. The CH<sub>3</sub> signal, however, appeared as a doublet in the presence of sufficient amount of acetone.

On hydrogen bond formation between the  $\text{C}=\text{O}$  (acetone) and  $-\text{OH}$  (methanol) the resident time of the OH proton in the hydroxyl group increases when coupling with methyl group becomes possible. The doublet signals for CH of  $-\text{CHOH}$  in benzoin and methyl mandelate therefore suggest bonding between OH and  $\text{C}=\text{O}$ , leading to the formation of a five membered chelate. This is also reflected in the

downfield shift of OH proton in CDCl<sub>3</sub> of benzoin (4.57  $\delta$ ) and of methyl mandelate (3.35  $\delta$ ), as compared to the OH proton in benzyl alcohol (2.43  $\delta$ )<sup>5</sup>.

The relative insensitivity of the OH proton signals to the changes in concentration further support the formation of intramolecular hydrogen bonding. The downfield shift of the OH proton in these compounds is not so great as in other chelate compounds, viz., salicylaldehyde, 2-hydroxy acetophenone and enols of  $\beta$ -diketones. Chelation in these compounds, therefore, appears to be weak. The weak chelation may be due to the absence of conjugation in the chelate ring, as found in the compounds mentioned above. The larger downfield shift of OH proton in benzoin as compared to that in methyl mandelate may be due to greater electron donating ability of the oxygen of the ketonic  $\text{C}=\text{O}$  than that of the ester  $\text{C}=\text{O}$ . The lone pair electrons on the oxygen of the ketonic  $\text{C}=\text{O}$  approach the OH proton more closely and repel greatly the electron which is in the neighbourhood of the hydrogen nucleus and therefore reduce the diamagnetic shielding of that nucleus by its own electrons and increase the paramagnetic deshielding<sup>6</sup>.

The influence of solvents on the chemical shifts of CH and OH protons of benzoin and methyl mandelate is rather interesting. The signals of both the protons remain as doublets indicating the absence of OH proton exchange as noticed in the presence of D<sub>2</sub>O, trifluoro acetic acid and methanol. With the increase in polarity of the solvent from CS<sub>2</sub> to DMSO the OH signals in general move to lower fields, the shifts being larger in  $n$ -donor solvents like DMSO, DMF and acetone.

Although the OH chemical shifts of benzoin and methyl mandelate are different in CDCl<sub>3</sub> and CS<sub>2</sub> both of them have almost the same value either in DMF and DMSO. This may happen when the OH groups of the two compounds are bonded to the same donor. In DMSO and DMF, the chelation may be ruptured and intermolecular hydrogen bonds may be formed with more basic solvent molecules as shown in Fig. 1.

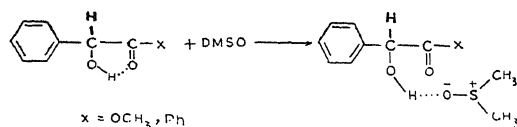


FIG. 1

This view is in agreement with an earlier finding<sup>7</sup> that the chelation in salicylaldehyde is disrupted with DMSO leading to the formation of an intermolecular hydrogen bonding.

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# ECOPHYSIOLOGY OF A HOST-PARASITE SYSTEM: EFFECT OF INFECTION OF A PARASITIC COPEPOD, *LERNAEA HESARGATTENSIS* ON THE OXYGEN CONSUMPTION OF THE FISH, *LEBISTES RETICULATUS* PETERS)

Work on the physiology of copepods, especially of the parasitic forms, is wanting<sup>1</sup>. A new species of a parasitic copepod *Lernaea hesargattensis*, infecting the cyprinodont fish *Lebistes reticulatus*, has been recently described<sup>2,3</sup>. The adult female parasites are found to be firmly embedded in the muscular tissue of the host and thus obtain their nutritional requirement.

One of the vital ecological factors affecting the survival of fish is the availability of oxygen in the aquatic habitats and the oxygen consumption of the fish is a direct index to its metabolic rate and food requirement<sup>4</sup>. Hence, in the present paper, the effects of infection of *L. hesargattensis* on the oxygen consumption of the fish *L. reticulatus* is described.

The test fish were collected from the fish ponds of Hesarghatta, near Bangalore. The fish were separated into the following experimental series: (1) Normal, (2) infected, with the parasite intact and (3) infected, with the parasites removed. Oxygen consumption of all these fish were estimated for males and females separately, using the modified Winkler's method<sup>5</sup>. In many of the infected fish, at the region of penetration of the parasite, certain tissue damage and inflammation around the area was observed. This reaction was local and did not extend beyond the area of infection. Similar local

reactions were recorded in salmonids due to infection by the leach *Piscicola salmonisica*<sup>6</sup>.

Table I represents the average values of oxygen consumed by males and females of the experimental series. A normal male fish consumed  $0.2685 \pm 0.051$  cc of oxygen/gram body weight/hour. During the same period the infected fish consumed as much as  $0.3314 \pm 0.139$  cc of oxygen/gram body weight.

TABLE I

*Effect of infection of the copepod parasite Lernaea hesargattensis on the oxygen consumption of male and female Lebistes reticulatus. Each value represents the mean of 6 experiments*

| Material                   | Sex    | Body weight (mg) | Oxygen consumed/g body weight/hour (cc) |
|----------------------------|--------|------------------|---|
| Normal                     | Male   | 196.80           | 0.2685                                  |
|                            |        | $\pm 13.83$      | $\pm 0.051$                             |
| Controls                   | Female | 264.27           | 0.7725                                  |
|                            |        | $\pm 22.67$      | $\pm 0.320$                             |
| Infected, with parasite    | Male   | 174.87           | 0.3314                                  |
|                            |        | $\pm 12.78$      | $\pm 0.139$                             |
|                            | Female | 329.05           | 0.8981                                  |
|                            |        | $\pm 26.92$      | $\pm 0.084$                             |
| Infected, without parasite | Male   | 114.58           | 0.2608                                  |
|                            |        | $\pm 8.94$       | $\pm 0.159$                             |
|                            | Female | 330.20           | 0.6614                                  |
|                            |        | $\pm 31.22$      | $\pm 0.332$                             |

The corresponding value for normal and infected females were  $0.7725 \pm 0.320$  and  $0.8981 \pm 0.084$  cc of oxygen/gram body weight/hour, respectively. The higher consumption by female *Lebistes*, despite a larger body weight when compared to males, may be due to the fact that these experiments were conducted during March 1973 when the laboratory water temperature was 32°C, five degrees more than the temperature at which the males were experimented (November, 1972).

In both male and female *Lebistes*, when the parasites were removed, the oxygen consumption dropped back almost to the normal values. In the males, on removal of the parasite, the fish consumed  $0.2608 \pm 0.159$  cc of oxygen/gram body weight/hour and in the females, the corresponding value was  $0.6614 \pm 0.332$  cc. Moreover, the oxygen consumed by the parasite alone (after it was removed from the host's tissue) was found to be negligible. Hence, this increased oxygen consumption of the infected *Lebistes*, irrespective of the sex of the fish, can be attributed to a 'stress reaction' due to parasitic infection of the copepod.

The percentage increase in the oxygen consumed, from the normal to infected males, was 20.42 while in females this increase was slightly less (16.30%).



A respiratory increase of 5% of the host's normal metabolic rate was observed in the sculpin *Myoxocephalus scorpius* infected by the leech, *Malmiana nuda*<sup>7</sup>. Even here, the increase was attributed to the increased rate of blood and tissue formation and to stress caused by the mechanical irritation of the feeding leeches. Thus, it may be surmised that though large respiratory increases may not be observed with smaller burdens of parasitic infection under natural conditions, oxygen deficient waters and/or heavy parasitic infections may be detrimental to the fish.

Further work on the ecophysiology of this host-parasite system is in progress.

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#### CHANGES IN CALCIUM AND PHOSPHATE LEVELS IN THE BONES OF BABY AND ADULT LORIS, *LORIS TARDIGRADUS*

THE extracellular constituent of bones is a system of collagen and calcium phosphate. The latter has a structure identical or similar to hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . It contains crystalline and amorphous fractions, the crystalline ones, increasing with age<sup>1</sup>. Concurrently, the Ca/P ratio shifts towards higher calcium values. The major constituent of bone is collagen. Its content amounts to approximately one-third of the total bone mass<sup>2</sup>.

Various substitutions and ion exchanges leading to the hydroxyapatite structure occur during maturation of human bones<sup>3,4</sup>. In man, it is also known

that the Mg content is highest in the bones of the youth which, as the age progresses, is replaced by the deposition of  $\text{Ca}^{5+}$ . This process of ion exchange is known as 'slagging'.

Whether such ion exchanges or slagging occurs with age in the slender loris, a prosimian, has been investigated in this paper.

Marrow free and oven dried humerus, femur and parietal bones of baby (1 month old) and adult (2 years old) were weighed and dissolved in 100 ml of 0.6 N HCl and the extract was analysed for calcium, magnesium, phosphorus and non-protein nitrogen. Calcium of the bone extract was precipitated as oxalate and titrated with standard potassium permanganate following the method of Clark and Collip<sup>6</sup>. After removal of calcium, magnesium of the extract was precipitated as magnesium ammonium phosphate according to Denis<sup>7</sup>. The resultant phosphate was estimated by Fiske-Subba Row method<sup>8</sup>. The bone extract was deproteinized with 5% trichloroacetic acid and the phosphorus of the protein-free filtrate was estimated according to Fiske-Subba Row<sup>8</sup>. The non-protein nitrogen (NPN) of the extract was determined by micro-Kjeldahl method as described by Oser<sup>9</sup>.

Analysis of bone extract revealed that the inorganic constituents varied with age and the bone. Calcium content significantly increased in the parietal bones but decreased in the femur during aging; phosphorus increased in the humerus in contrast to the femur and the parietal but the nitrogen content decreased in general, on aging.

In mammals (including man), the magnesium content of the bone is very low<sup>5</sup>, but in baby and adult loris it is higher than in other mammals. Magnesium, however, decreased in all bones on aging (Table I). In loris, blood also contains a high magnesium content<sup>10</sup> which might account for the high magnesium content in the bone. Long bones showed a lower and the parietals a higher phosphorus content in comparison with other mammals.

The theoretical molar ratio (Ca/P) for hydroxy apatite is 1.667 which differed with age and individual bones of loris (Table II); it increased on aging in the parietals and the femur in contrast to humerus. The ratios relatively higher than the theoretical ratio (Table II) probably indicate that calcium occurs in forms other than hydroxy apatite also. Molar ratios Ca/P, Mg/P and Ca/Mg (Table II) indicated that calcium might exist in different forms like calcium carbonate, in addition to its occurrence as hydroxy apatite. The high magnesium and low phosphorus contents of baby bones suggest the occurrence of a low hydroxy apatite content in young bones.

TABLE I

Bone composition in *L. tardigradus* (values are mean  $\pm$  S.D. of samples from 4 animals)

| Mg/g dry weight | Baby            |                 |                 | Adult           |                 |                 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Humerus         | Femur           | Parietal        | Humerus         | Femur           | Parietal        |
| Calcium         | 256 $\pm$ 9     | 234 $\pm$ 6     | 175 $\pm$ 8     | 254 $\pm$ 11    | 211 $\pm$ 5     | 246 $\pm$ 7     |
| Phosphorus      | 60 $\pm$ 4      | 134 $\pm$ 8     | 761 $\pm$ 11    | 109 $\pm$ 13    | 75 $\pm$ 6      | 576 $\pm$ 14    |
| Magnesium       | 109 $\pm$ 2     | 109 $\pm$ 2     | 64 $\pm$ 2      | 18 $\pm$ 2      | 13 $\pm$ 2      | 27 $\pm$ 2      |
| NPN             | 2.28 $\pm$ 0.11 | 7.46 $\pm$ 0.16 | 4.44 $\pm$ 0.13 | 0.26 $\pm$ 0.08 | 0.16 $\pm$ 0.06 | 2.46 $\pm$ 0.09 |

TABLE II

Molar ratios of the inorganic components of bones in *Loris tardigradus*

| Molar ratio | Adult   |       |          | Baby    |       |          |
|-------------|---------|-------|----------|---------|-------|----------|
|             | Humerus | Femur | Parietal | Humerus | Femur | Parietal |
| Ca/Mg       | 2.25    | 2.56  | 13.92    | 3.84    | 3.42  | 4.37     |
| Ca/P        | 3.01    | 3.65  | 0.55     | 5.51    | 2.36  | 0.32     |
| Mg/P        | 0.13    | 0.13  | 0.04     | 1.43    | 0.63  | 0.07     |

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### OBSERVATIONS ON THE SCALES OF THE SHORT-FINNED EEL, *ANGUILLA BICOLOR* McCLELLAND AND THEIR UTILITY IN AGE DETERMINATION

It is well known that scales of fishes are of value in determining the age and growth of fishes. Several authors (Lee, 1920; Van Oostern, 1929; Walford and Mosher, 1943; Seshappa and Bhimachar, 1951; Jhingran, 1957; Seshappa, 1958 and Rao, 1961) have determined the age of various species of fishes based on the number of rings found on the scales. Pantulu (1956) has stated that scales could be used in estimating the age and not growth of the eel, *Anguilla bengalensis* Gray. In 1971 experimental eel culture was undertaken at the Regional Centre of Central Marine Fisheries Research Institute, Mandapam Camp, to develop a suitable method of culturing the short-finned eel, *Anguilla bicolor* McClelland. During the course of the experimental culture the scales of the cultured eels were studied to ascertain whether there is any correlation between the number of rings present on them and the age of eels. The results obtained in this study are reported here.

About 200 elvers of an average length of 100 mm were collected near the closed sluice gates of Srivaikundam anicut on the river Tambraparni near Tuticorin in October 1971, transported to Mandapam Camp and reared in running water tanks. They were fed daily twice with minced clam meat and fish flesh. The water temperature in the experimental tank varied between 28° and 30° C. The size attained by the cultured eels at the end of one year exhibited a wide range of 125 mm to 500 mm and the average size was 350 mm in total length and 106 gm in weight. For the present study scales were examined from cultured eels measuring 128 mm, 153 mm, 283 mm, 363 mm, 388 mm, and 500 mm in total length. Scales were removed from the area midway between anus and tip of tail on either side of the lateral line. 10 to 20 scales were examined from each eel. In this investigation scales having maximum number of rings alone were taken into considera-

tion as they are believed to represent the correct age of eels (Gemzoe, 1904; Frost, 1945 *a* & *b*).

The scales of eels are microscopic, very thin, flat and elongate-oval in shape and are embedded in the skin at right angles to each other (Pl. 1 A). They do not overlap but are "placed in individual sacs in the dermal tissue with no connection with the epidermal coverings" (Waly, 1940). The scales are composed of concentric rows of oval, round or polygonal head-like loculi.

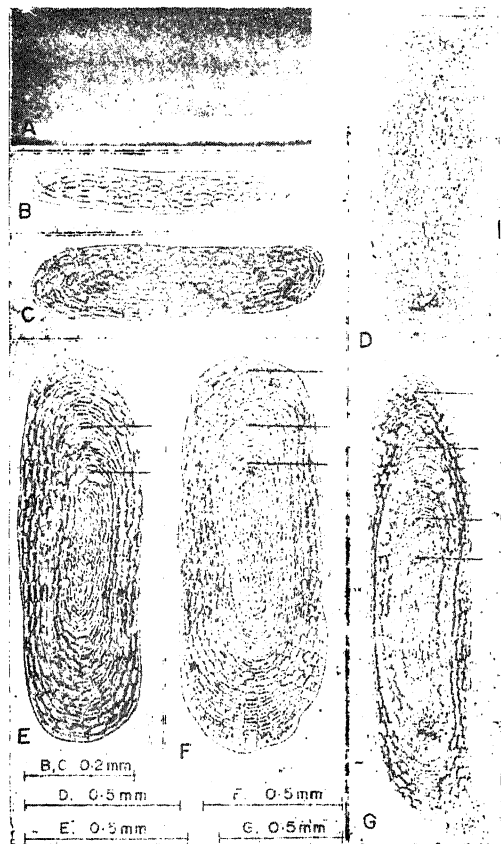


PLATE 1. A, A portion of the lateral body surface of *Anguilla bicolor* McClelland, showing the disposition of scales. The lateral line also may be seen in the photograph; B, Scale of 128 mm long eel showing no ring; C, Scale of 153 mm long eel showing no ring; D, Scale of 283 mm long eel showing one ring; E, Scale of 363 mm long eel showing two rings; F, Scale of 388 mm long eel showing three rings; G, Scale of 500 mm long eel showing four rings.

Photomicrographs of scales, collected from eels of different sizes are shown in Plate 1 B-G. It may be seen that there are no rings on the scales collected from 128 mm and 153 mm long eels, whereas in 283 mm, 363 mm, and 500 mm long eels the

scales have one, two, three and four rings respectively. These results may lead to the conclusion that 283 mm, 363 mm, 388 mm, and 500 mm long eels are one, two, three and four years old respectively. It may be pointed out here that for this study scales were collected from eels reared in running water tank and though the eels ranged from 128 mm to 500 mm in total length, all of them were of the same age, as they had grown to these sizes from elvers during one year period. But their scales show varying number of rings. These observations clearly indicate that there is no relationship between the number of rings found on the scales and the age of eels. In the light of the above finding, the view so far held that the rings found on scales are annual in character and are indicative of age of fish, does not hold good for *A. bicolor*. In the present investigation the eels were reared in an environment where there were no marked changes either in the availability of food or temperature, which are normally believed to be responsible for formation of rings on scales. Therefore the causative factor for the formation of rings on scales is not clear.

Based on length frequency data and scale readings Pantulu (1956) has estimated age of *Anguilla bengalensis*. According to him the respective average length of I to V year old eels, as estimated from length frequency are 210 mm, 265 mm, 331 mm, 441 mm and 507 mm and as calculated from scale readings are 183.5 mm, 249.5 mm, 340 mm, 447 mm and 516.6 mm. Since there is a close agreement in the estimates of age calculated by the two methods, he stated that scales could be utilized for estimating the age of *A. bengalensis*.

The present experimental culture of *A. bicolor*, an allied species of *A. bengalensis*, has clearly shown that there is a wide range in the length of eels of same age and that there is no relationship between the number of rings present on the scales and age of eels. Bertin (1956) has also stated that measurement of length or weight cannot be used to determine the age and growth of an eel, as it would risk an error of one to five years either way. Therefore it may be stated that in the case of eels any estimate of age arrived at by length frequency method and scale readings is likely to be erroneous. The only reliable method of determining the age of eels is by making direct observations on the rate of growth through rearing.

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### COMMON ANTIGENS IN HOST-PARASITE RELATIONSHIP\*

It has been shown in mammalian systems, that antigenic closeness between a host and a parasite, leads to a stable host-parasite relationship (host 'tolerance') while antigenic disparity leads to host resistance to the parasite (host 'intolerance')<sup>1-3</sup>. Although the plant does not produce an immune system similar to that in animals, the concept that common antigens between host and parasite might have a role in disease development found some support in plant pathology as well<sup>4,5</sup>. The present investigation is an attempt to see if this concept is applicable in vascular wilt of cotton.

Experiments were designed to look for possible common antigens between two species of cotton, *Gossypium arboreum* L. and *G. hirsutum* L. and a virulent Indian strain of *Fusarium vasinfectum* Atk. Earlier work has shown that this strain is highly pathogenic to *G. arboreum* but not to *G. hirsutum* although it infects and colonizes both to varying degrees<sup>6</sup>. In order to exclude errors owing to non-specific reactions in the final results, the following fungi and plants were included for reference; *Fusarium solani* (Mart.) App. et Wr., *F. culmorum* (W.G. Sm.) Sacc. and *Pyricularia oryzae* Cav. none of which are known parasites of cotton, and *Abelmoschus esculentus* (L.) Moench, and *Phaseolus mungo* L. plants that are not known to be infected by *F. vasinfectum*.

The extraction of the fungal antigens was as described in an earlier paper<sup>7</sup>. For host antigens the initial steps of extraction were the same as outlined by DeVay and co-workers<sup>4</sup>. The material was extracted under liquid nitrogen using polyvinyl pyrrolidone and sodium ascorbate with a pestle and mortar. Further homogenization was done in a VirTis homogeniser. The extractant used was phosphate-buffered saline at pH 7.2. The antigens were purified further by the same procedure as

for fungi<sup>7</sup>. The antigens mixed with Freund's complete adjuvant were administered intramuscularly in a course of six injections to white rabbits (each weighing approximately 1.5 kg). Antisera were collected on 23rd day by bleeding the ear veins and stored at 0° C with merthiolate as preservative. The antisera against the two species of cotton were tested by the agar-gel double diffusion method with their antigens in homologous and heterologous reactions. The antigens of *A. esculentus* and *P. mungo* were also allowed to react with the two antisera. *G. arboreum* formed three lines of precipitation in homologous reactions and two in heterologous reaction with *G. hirsutum* while the latter showed two antigens in homologous as well as heterologous reactions. *A. esculentus*, which belongs to the same family as cotton, shared an antigen with both the species of cotton. *P. mungo* produced no precipitation with either species of cotton.

Using the same technique, the antisera and antigens of the two species of cotton were tested against the antigens and antisera, respectively, of all the test fungi. The antisera of the two species of cotton on reacting with the antigens of *F. vasinfectum*, or the antisera of the latter on reacting with the antigens of the former two, formed a single precipitin band indicating the presence of a common antigen (Fig. 1). However, such a result

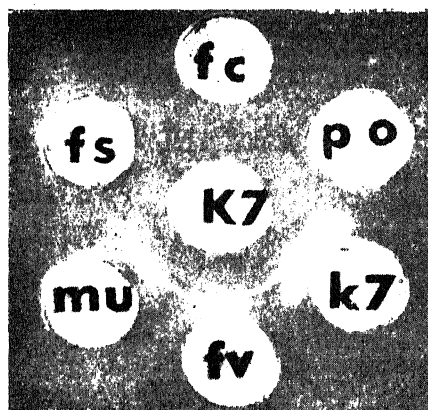


FIG. 1. Antigen of *G. arboreum* in the central well (K7) reacting with homologous antiserum (k7) and with heterologous antisera of *G. hirsutum* (mu), *Fusarium vasinfectum* (fv), *F. culmorum* (fc), *F. solani* (fs) and *Pyricularia oryzae* (po).

was not observed in any of the reciprocal reactions involving the other test fungi and the two cotton species, or in those between *F. vasinfectum* and the other plants.

The results of this investigation indicate the presence of a common antigen between the pathogen *F. vasinfectum* and its hosts. However, this

concept of a common antigen between a host and parasite as related to disease development ought, in our opinion, to place emphasis on *parasitism* rather than on *pathogenesis*. The intrinsic ability of the Indian strain of *F. vasinfectum* to infect and colonize (*i.e.*, to parasitize) both the species of cotton, although disease manifestation is seen only in *G. arboreum*, is compatible with this idea. University Bot. Lab.,  
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### EFFECT OF DIFFERENT STORAGE TEMPERATURES ON KEEPING QUALITY OF AVOCADO PEAR FRUITS

It had been known for a long time that low temperature is a good means for keeping fruit after harvest. Low temperature disorders are also a determining factor for storage ability of fruits. Prolonged storage of avocado at 5° C, however, resulted in a declining rate of CO<sub>2</sub> production upon removal to 15° C. Chilling symptoms appeared along with the disturbance of the climacteric pattern (Biale 1941; Pratt and Biale 1944). The lowest temperature at which a rise of respiration was noted in the fuerte avocado was 7.5° C (Biale, 1946).

Mustard (1952) found that different varieties of avocados differ in its sensitivity to chilling injury. Campbell (1960) found that chilling injury occurred in mature pollack avocados stored at 35, 40, 45 and 50° F for 19 to 22 days. Biale and Young (1962) observed that in avocados at 30° F and 35° F, the fruit does not ripen but the tissue darkens.

Aharoni *et al.* (1968) stored avocado fruit at gradually decreasing temperatures, and found that the climacteric peak and softening appeared at the same time in the fruit stored at 12° (prior to the fruit stored at 8° and subsequent to that stored at 14°, 15° and 17° C). The purpose of this work is to study the effect of various storage temperatures on the quality of avocado fruits.

### Materials and Methods

Freshly harvested mature fruits of Duke avocado were sorted out for size, shape, firmness to obtain uniform samples. The fruits were washed with water, then dipped for 5 minutes in solution of 5% borax as a fungicidal treatment and the samples were placed in fiberboard boxes using three carton boxes for each treatment as replicates, then cooled to the required temperatures.

Visual evaluation of the quality was made every 2 days according to the numerical quality score as described by Abdel Kader *et al.* (1968). At the same time unusable fruits were discarded and the causes of decay were recorded. At every sorting time, chilling injury symptoms were observed and noted, and also pathogenes were identified. Experiments were repeated 2 times and the average results are presented.

### Results and Discussion

The relative effects of storage temperatures on quality and decay percentage of "Duke" avocado fruits is illustrated graphically in Fig. 1. Fruits held at room temperature were the first to deteriorate. Their quality loss was mainly a result of senescence as was evidenced by the loss of firmness and the development of black colour. Fruits held at 15° C followed control fruits in their deterioration.

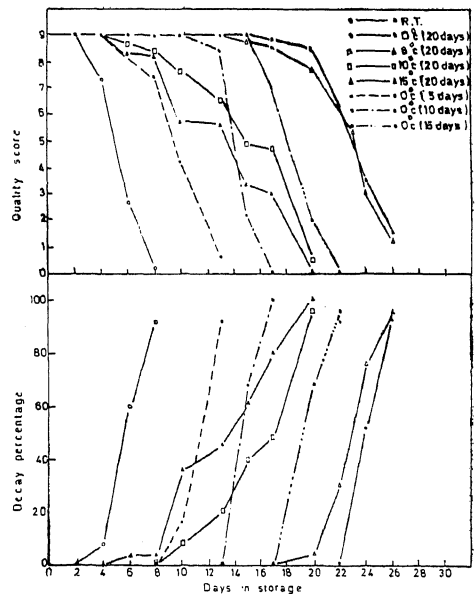


Fig. 1. Effect of temperature on quality and decay percentage of "Duke" avocado fruits during storage.

Avocado fruits that were subjected to 0° C for 5, 10, 15 and 20 days did not show any decay or

deterioration symptoms and also were in good quality during storage at 0° C. However, after removal to room temperature, the rate of decay was so rapid that all fruits were decayed within 2 days. No chilling injury symptoms were noticed on fruits that were stored at 0° C or 5° C before removal to room temperature. After transfer to room temperature the flesh discoloured and some black areas appeared on the skin without developing the texture, characteristic of avocado. In the severe cases the fruits do not ripe at all.

The rate of deterioration at 0° and 5° was of the same order for a period of 20 days. On the other hand, fruits held at 10° C exhibited a faster rate of deterioration although no chilling injury symptoms were observed on them. Fruits stored at 10° C can be kept in a good condition at this temperature for approximately 2 weeks and still have several days for marketing purposes. Fruits held at this temperature deteriorated to an unsalable quality only after 18 days as compared with 6 days for those held at room temperature.

The rate of decay was lower in fruits held at 10° C than those held at room temperature, they reached 50% decay after 17 and 6 days in storage respectively.

Generally, at temperatures much below 10° C. Duke avocado fruits were subjected to the symptoms of chilling injury. Thus, a temperature of 10° C seemed to be the most suitable for storage of Duke avocado fruits.

Avocado fruits were attacked by several pathogens as they became weak as a result of senescence or chilling injury. The identified pathogens included: *Fusarium* sp., *Alternaria* sp., and *Penicillium* sp. in some fruits held at room temperature.

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## EMBRYO DEVELOPMENT IN *EUPHORBIA PEPLUS* L.

EMBRYOGENY in the family Euphorbiaceae is of particular interest in view of the reports of widespread occurrence of five major types of embryo development<sup>2-6</sup>. Despite this fact, details of normal embryogeny are available for restricted members. Within the genus *Euphorbia* L., while most of its species, so far embryologically known, display embryogeny conforming to the Euphorbia variation of Onagrad type, *Euphorbia corrigioloides*<sup>7</sup> follows Lotus variation. The report of Scabiosa variation of the Piperad type of embryo development in *Euphorbia rothiana*<sup>8</sup> has since been confuted and shown to conform to the Euphorbia variation of the Onagrad type<sup>3,9</sup>. But in *Euphorbia peltata*<sup>5</sup> and *Euphorbia preslii*<sup>10</sup> the embryo development follows the Chenopodiad and the Piperad type respectively. In view of this variance in embryogeny and since details of description are available for only a few species, the authors deliberate worthwhile studying the embryo development in some more species of *Euphorbia*, and also in the doubtful cases. This communication is concerned with the details of embryo development in *Euphorbia peplus* L.

The zygote (Fig. 2) undergoes a short period of rest and divides when endosperm is 10-nucleate (Fig. 1). Its first division is transverse forming a terminal cell *ca* and basal cell *cb* (Figs. 1, 3). The cell, *ca*, divides vertically (Fig. 4), while *cb* divides transversely to procreate *m* and *ci* (Figs. 5-7) thus producing a 4-celled proembryo (Fig. 6) with cells disposed in three tiers. At times the division of *cb* precedes that of *ca* (Fig. 5) but in our material these cells have never been found to divide synchronously (Figs. 4, 5). The two cells of *ca* divide by another vertical wall in a plane at right angles to the first division (Fig. 7) resulting in a quadrant; the cells of which later divide transversely resulting in octants disposed in two tiers, *l* and *l'* (Figs. 8, 9). The cell, *m*, after longitudinal and transverse divisions becomes the hypophysis (Figs. 8-16). With further differentiation the embryo passes through the globular and heart-shaped stages before it becomes fully differentiated and mature (Figs. 14-18). The cells of the tier *l* give rise to the stem apex (*prt*) and the cotyledons (*pco*), while the derivatives of *l'* function to form the hypocotyledonary region (*phy*) and initials of the central cylinder of the stem (*icc*), *m* to the root cortex (*iec*) and root cap (*co*) and *ci* remains as 1-celled suspensor (*s*).

The course of events taking place in the different tiers of the proembryo is as follows:

In each cell of the terminal tier *l*, an oblique wall is laid down initiating two cells, the inner and

outer (Fig. 10). The former cells on division both transversely and longitudinally over and again differentiate into the plumule, while the latter cells constitute the initials for cotyledons. Concomitantly the cells of the tier *l'* divide periclinally to demarcate the dermatogen (Figs. 11, 12) which likewise develops in the tiers *l* and *m* (Figs. 12–14).

The derivatives of *l'* ultimately contribute to the hypocotyledonary region and the initials of central cylinder of the stem (Figs. 12–16). Subsequent divisions in the inner cells of *l'* demarcate the plerome and perilem (Figs. 14–16).

The basal cell *cb* of the 2-celled proembryo divides by a transverse wall to form *m* and *ci*. The tier *m* which is 1-celled at the 3, 4 and 6-celled stages of the proembryo (Figs. 5–7) functions as a hypophysis initial, divide by a vertical wall forming two juxtaposed cells (Figs. 8–10). The two cells further divide vertically to engender a group of four cells (Fig. 11). Subsequently these divide periclinally to initiate four peripheral cells and four inner cells. The four peripheral cells complete dermatogen of the root tip (Figs. 12–16) as also represent the primordium of the root cap. The inner cells on further division organise two tiers of four cells each (Figs. 13, 14) which constitute the initials of root cortex and finally form the root tip.

The tier *ci* remains as a 1-celled suspensor (Figs. 5–16) which is lost during subsequent development and growth of the embryo in seed (Figs. 17, 18).

The mature embryo is dicotyledonous with well-developed shoot apex, root and vascular supply (Fig. 18).

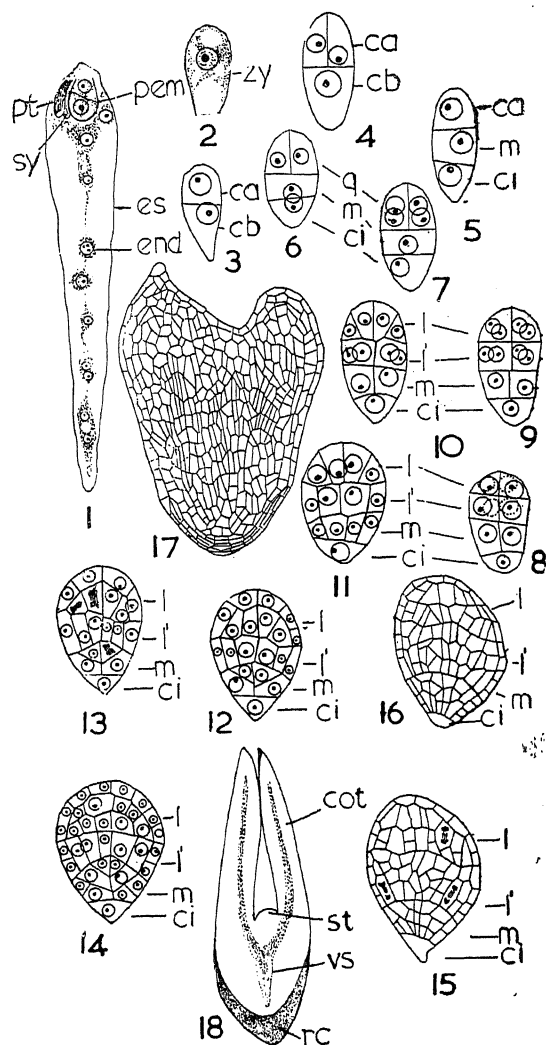
From the foregoing account it is obvious that the terminal cell, *ca*, of 2-celled proembryo divides by a vertical wall and the basal cell, *cb*, has merely a restricted role in the formation of the embryo proper. Therefore, the embryo development in *Euphorbia peplus* follows the Onagrad type of Johansen<sup>4</sup> and keys out to the Euphorbia variation, as the 6-celled proembryo is disposed in three tiers. Under Souéges scheme (*vide* Crété)<sup>1</sup> it can be matched with the Megarchtype IV, series A in First Period.

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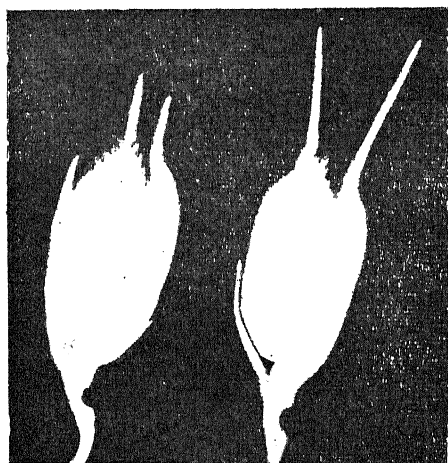


FIGS. 1–18. Fig. 1. Embryo sac with 2-celled proembryo and 10-nucleate endosperm,  $\times 200$ . Figs. 2–14. Stages in the development of the embryo,  $\times 200$ . Figs. 15–17. Advanced stages in the development of embryo,  $\times 300$ . Fig. 18. L.S. Embryo showing two cotyledons, stem tip, root tip, root cap and vascular supply,  $\times 30$ . (*cot*, cotyledons; *end*, endosperm; *es*, embryo sac; *pem*, proembryo; *pt*, persistent pollen tube; *rc*, root cap; *st*, stem tip; *sy*, synergid; *vs*, vascular supply.).

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### DOUBLE AWNING IN TETRAPLOID RICE

THE present note records the development of awns on both lemma and glume (sterile lemma) in an intervarietal tetraploid grown at Central Rice Research Institute, Cuttack. This variation in awning was expressed in about five percent of the spikelets of the plant. The following observations were recorded in these spikelets: The mid-nerve of the lemma and glume on palea side extended into awns. The awns were either short or medium in length while those developing from the lemma in normal spikelets were always long. The awn bearing glume was broad and 3-nerved enclosing about 3/4 length of the palea. The glume on the lemma side was short and was awnless. The awns developing from the lemma and glume appeared similar. Invariably, one extra glume was also present on the palea side (Fig. 1).



Subsequent studies showed that the variation was teratological in nature. It may be inferred that the nerves of the glumes are also capable of extending into awns. This has been confirmed by Prasad

(Personal communication) who observed a diploid variety where the awn developed from sterile glume. Earlier, the nerves of the lemma and palea were only considered capable of developing into awns<sup>1</sup>.

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### VOLATILE SPOROSTATIC FACTORS OF ASPERGILLI AND THEIR ROLE IN SOIL FUNGISTASIS

STUDIES on volatile inhibitors/stimulators have gained momentum in the last few years and several review articles have appeared recently<sup>3-6,7</sup>. Besides their involvement in general growth and development, volatile inhibitors have been implicated in ecology of fungi particularly in soil fungistasis<sup>1,5</sup>. Earlier works in this laboratory have provided valuable data in this direction<sup>8-10</sup>. In continuation of these efforts, production of volatiles by *Aspergilli* was investigated and efforts have been made to correlate these results with soil fungistasis.

Eighteen species of *Aspergillus* used in this study were procured from the Mycology Laboratory, University of Saugar (Table I). Spores of *Alter-*

TABLE I  
Production of volatile sporostatic factors by  
*Aspergilli* in vivo and in vitro

| Antagonist                  | % inhibition of test spores |          |                           |          |                           |          |
|-----------------------------|-----------------------------|----------|---------------------------|----------|---------------------------|----------|
|                             | <i>Alternaria solani</i>    |          | <i>Rhizopus nigricans</i> |          | <i>Trichoderma viride</i> |          |
|                             | In vivo                     | In vitro | In vivo                   | In vitro | In vivo                   | In vitro |
| <i>Aspergillus candidus</i> | 0                           | 47       | 17                        | 51       | 13                        | 12       |
| <i>A. fischeri</i>          | 0                           | 42       | 25                        | 61       | 26                        | 26       |
| <i>A. flavus</i> I          | 0                           | 50       | 51                        | 83       | 18                        | 16       |
| <i>A. flavus</i> II         | 0                           | 64       | 49                        | 95       | 20                        | 81       |
| <i>A. flavus</i> III        | 92                          | 49       | 95                        | 71       | 92                        | 20       |
| <i>A. fumigatus</i>         | 64                          | 55       | 100                       | 89       | 63                        | 50       |
| <i>A. glaucus</i>           | 10                          | 35       | 14                        | 48       | 12                        | 10       |
| <i>A. nidulans</i>          | 0                           | 52       | 71                        | 60       | 86                        | 51       |
| <i>A. niger</i>             | 50                          | 89       | 100                       | 91       | 89                        | 95       |
| <i>A. ochraceous</i>        | 0                           | 19       | 86                        | 49       | 40                        | 11       |
| <i>A. oryzae</i>            | 51                          | 30       | 100                       | 49       | 87                        | 25       |
| <i>A. sclerotiorum</i>      | 0                           | 40       | 19                        | 60       | 14                        | 13       |
| <i>A. sydowii</i>           | 0                           | 11       | 21                        | 40       | 30                        | 31       |
| <i>A. terreus</i> I         | 63                          | 55       | 100                       | 83       | 74                        | 64       |
| <i>A. terreus</i> II        | 51                          | 79       | 100                       | 93       | 70                        | 51       |
| <i>A. terreus</i> III       | 65                          | 66       | 100                       | 91       | 57                        | 52       |
| <i>A. terreus</i> IV        | 75                          | 51       | 100                       | 61       | 87                        | 49       |
| <i>A. ustus</i>             | 0                           | 10       | 70                        | 80       | 81                        | 0        |



TABLE II  
Time course production of volatiles by selected *Aspergilli*

| <i>Aspergillus</i> spp. | % inhibition of test spores |    |    |    |                           |    |    |    |                           |    |    |    |    |
|-------------------------|-----------------------------|----|----|----|---------------------------|----|----|----|---------------------------|----|----|----|----|
|                         | <i>Alternaria solani</i>    |    |    |    | <i>Rhizopus nigricans</i> |    |    |    | <i>Trichoderma viride</i> |    |    |    |    |
|                         | Time (Days)                 |    |    |    |                           |    |    |    |                           |    |    |    |    |
|                         | 2                           | 4  | 6  | 8  | 2                         | 4  | 6  | 8  | 2                         | 4  | 6  | 8  |    |
| <i>A. flavus</i> II     | ..                          | 31 | 12 | 10 | 49                        | 38 | 41 | 41 | 55                        | 15 | 24 | 71 | 55 |
| <i>A. niger</i>         | ..                          | 0  | 31 | 51 | 38                        | 15 | 19 | 43 | 51                        | 24 | 13 | 40 | 49 |
| <i>A. terreus</i> I     | ..                          | 10 | 13 | 21 | 51                        | 11 | 29 | 61 | 71                        | 14 | 16 | 73 | 71 |
| <i>A. terreus</i> II    | ..                          | 0  | 10 | 20 | 22                        | 30 | 16 | 58 | 65                        | 23 | 26 | 73 | 76 |
| <i>A. terreus</i> III   | ..                          | 5  | 10 | 21 | 45                        | 5  | 30 | 39 | 69                        | 34 | 39 | 45 | 60 |

*naria solani* (Ellis and Martin), *Rhizopus nigricans* Ehrenberg, and *Trichoderma viride* Pers ex. Fries were used as test organisms. Czapek's Dox (CDA) and potato dextrose agar (PDA) media were used for maintaining *Aspergilli* and the test fungi, respectively. Spore suspension of the test fungi was prepared from 4–8 day old cultures, whereas inoculum of the antagonists was 10–15 day old. Routine evaluation of volatile inhibitors was made with a cellophane agar-disc technique<sup>9</sup>; 2% glucose was incorporated in the discs to facilitate germination of test spores. Individual species of *Aspergillus* was either mixed in soil (*in vivo*) or inoculated in CDA (*in vitro*). Sterile cellophane circle was placed over it to avoid contamination of agar discs and a sterile U-tube was kept over it. Agar discs were placed on a sterile glass slide which in turn was supported on the U-tube. The upper lid of the plate was placed back in its original position and the whole assembly was incubated for 10 days. Spore suspension of test fungi was placed on these pre-activated discs and they were further incubated for 8–12 hr. Germination counts for at least 150 spores were made and from this data percentage inhibition was calculated. Sterilized uninoculated soil or CDA was used in the control set of experiments.

In the experiment involving inoculation of sterilized soil by *Aspergilli*, spores of *R. nigricans* were most strongly inhibited (Table I). This was particularly noticeable when antagonists were, *A. fumigatus*, *A. oryzae*, and strains of *A. terreus*; spores of *Alternaria* and *T. viride* were only weakly inhibited: Half of the *Aspergilli* used in this study were ineffective against spores of *Alternaria*. It was visually observed that better fungal growth in soil often resulted in higher inhibition of the test fungus. In contrast to the observations of Singhai<sup>10</sup>, *A. flavus*

III was noted to be a potent inhibitor of all the three test fungi.

Inhibition of test spores was also evident when *Aspergilli* were inoculated individually in CDA. *Aspergillus niger* appeared to be more potent than *A. flavus* III (Table I); inhibition by *A. flavus* I was only slightly weaker. *Aspergillus fumigatus* and *A. terreus* could also bring about considerable inhibition of test spores. Some difference in the performance of antagonists *in vivo* and *in vitro* can be observed. It would appear that a change in the substrate tempers with the growth and thereby results in variable production of volatiles in soil and CDA.

A time-course study of volatile production by *A. flavus* II, *A. niger* and strains of *A. terreus* was undertaken *in vitro* (Table II). *Trichoderma viride* appeared to be most sensitive to the volatiles of these five antagonists since no less than 50% inhibition was noted against them after 8 days of incubation (Table II). Maximum inhibition by *A. flavus* II (71%) against this test fungus was noted after 6 days. *Rhizopus nigricans* ranked second in the order of its sensitivity and exhibited 50–70% inhibition after 8 days of incubation. For these two test fungi some degree of inhibition was evident even after 2 days of growth (Table II); spores of *Alternaria*, however, exhibited none or very little inhibition at this stage.

Further confirmation of the volatile nature of sporostatic factors was obtained by employing two other test procedures, viz., soil emanation agar<sup>5</sup> and paired Petri plates<sup>2</sup>. Inhibition of test spores by *A. flavus* II, *A. niger*, and *A. terreus* I and II was strongly evident in these tests as well (Table III). When the preactivated discs, with strongly inhibited spores, were removed to an ordinary moist chamber the germination percentage increased (Table III,

TABLE III

A comparison of volatiles of aspergilli produced under two different techniques

|                  |    | % germination of test spores |    |              |    |           |     |                              |    |              |    |           |     |
|------------------|----|------------------------------|----|--------------|----|-----------|-----|------------------------------|----|--------------|----|-----------|-----|
| Aspergillus spp. |    | Emanation Agar Technique     |    |              |    |           |     | Paired Petri Plate Technique |    |              |    |           |     |
|                  |    | A. solani                    |    | R. nigricans |    | T. viride |     | A. solani                    |    | R. nigricans |    | T. viride |     |
|                  |    | I                            | II | I            | II | I         | II  | I                            | II | I            | II | I         | II  |
| A. flavus II     | .. | 33                           | 87 | 37           | 82 | 28        | 95  | 34                           | 88 | 25           | 76 | 52        | 97  |
| A. niger         | .. | 24                           | 92 | 21           | 83 | 20        | 93  | 50                           | 92 | 21           | 79 | 49        | 95  |
| A. terreus I     | .. | 50                           | 91 | 21           | 78 | 18        | 97  | 25                           | 97 | 19           | 83 | 40        | 100 |
| A. terreus II    | .. | 48                           | 95 | 19           | 89 | 17        | 100 | 22                           | 94 | 20           | 81 | 22        | 88  |

I—Spore germination on pre-activated agar discs placed in the experimental chamber for 10 days.

II—Stimulation of spore germination after removing the agar discs to an ordinary moist chamber.

column II). This suggested an extremely labile nature and fungistatic action of the inhibitory principle(s). Thus soil inhabiting fungi of the genus *Aspergillus* appear to be strong producers of volatile sporostatic factors which have a positive role in ecology of soil fungi especially in the realm of soil fungistasis.

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#### BRYOPHYTES AS ROCK BUILDERS—SOME CALCICOLE MOSSES AND LIVERWORTS ASSOCIATED WITH TRAVERTINE FORMATION AT SAHASRADHARA, DEHRA DUN

ALTHOUGH tufa-building bryophytes and other plants have aroused a good deal of interest among ecologists of various countries of Europe and America<sup>1-3</sup>, no such work has so far been carried out in India. Further the need for such studies is emphasized by the ecosensitive character of bryophytes which are the most active agents in

building a biogenic rock like calcareous tufa or travertine. Accordingly the present study of the bryophytes associated with travertine at Sahasradhara was undertaken to fill up this long standing gap in our knowledge. Out of active tufa-building bryophytes, *Barbula gracilentia*, *Bryum cellulare*, *Hymenostyliella involuta*, *Vesicularia montagnei* and *Asterella maculata* are being reported for the first time as tufa-builders. The pottiaceous *Hymenostyliella involuta* previously known only from the Luzon Island in the Philippines (Bartram)<sup>4</sup> has recently been reported from Dehra Dun (Vohra)<sup>5</sup>. The tufaceous nature of this moss is, however, not mentioned in either of the two above-mentioned reports. In the present study, it forms an epiphytic association with the filamentous blue-green alga *Petalonema alatum*, var. *indicum* colonizing the bare boulders of limestone and all tufaceous seeps. Among other associates of tufa formation are calcareous algae like *Chroococcus*, *Gloeothece*, *Merismopedia*, *Petalonema alatum* Berk. var. *indicum* Rao, *Cladophora*, *Nitzschia*, *Pinnularia*, *Tabellaria* and *Cosmarium* and even among these *Merismopedia*, *Petalonema alatum* var. *indicum*, *Pinnularia* and *Tabellaria* are being reported as participants in tufa formation for the first time. Besides, the above forms, a few angiosperms like *Colocasia esculenta* (Linn.) Schott. var. *stolonifera*, *Didymocarpus pedicellata* R.Br., *Epipactis consimilis* Wall., *Eupatorium glandulosum*, H.B. & K., *Itea nultans* Royale and *Primula floribunda* Wall. have also been found to help in building tufaceous rock although they do so only when their exposed parts become covered with an algal or bryophytic felt.

The present study has, to some extent, helped in resolving the controversy about the physico-chemical factors involved in travertine deposition. In particular it shows that biological factors like thick

algal felts and dense moss polsters induce the development of travertine mainly by providing spongy surfaces which can absorb, retain and expose copious thin films of water for effective evaporation and consequent diffusion of  $\text{CO}_2$  from the calcareous spring water thus causing the precipitation of  $\text{CaCO}_3$  in the form of travertine. The mineral matter (calcite) hardens round the mosses taking the mould of their forms and these and other plants act as nuclei around which travertine is deposited.

The vigorous growth rate of these organisms (algae and mosses) seems to exceed the rate of carbonate deposition so that the process of getting cemented below and growing above is continued and the tufa also "grows" up.

These observations generally confirm the views held by Emig<sup>6-7</sup> about the role of plants in the mechanism of travertine deposition at Oklahoma. They also point to the calcicole character of the tufaceous bryophytes, algae and other plants and their indicator value. It would indeed be worthwhile trying some of the angiosperms growing on tufa for cultivation in alkali soils.

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#### X-RAY INDUCED ANEUPLOIDY IN *CAPSICUM ANNUM*

THE induction of aneuploidy has been useful in breeding crop plants. Experiments in bread wheat<sup>1</sup> demonstrate the possibility of transferring beneficial chromosomes from comparatively less useful types via aneuploids. Breeding for aneuploids with a view to identifying the chromosomes and linkage groups has also been described in other crops such as barley<sup>2</sup>, tomato<sup>3</sup> and groundnut<sup>4</sup>. The occurrence of aneuploids in members of Solanaceae was reported by Hermesen<sup>5</sup>. The present study concerns

the behaviour of the aneuploid chromosome in meiosis from X2 generation obtained from X-ray irradiated seeds following presoaking.

The seeds of *Capsicum annum* ( $2n = 24$ ) obtained from National Seed Corporation of India, Warangal Branch, were soaked for 6 hours and exposed to radiation at 4000 rads. Meiosis in these plants was studied after fixing the flower buds in acetic acid-alcohol (1:3) and squashing the anthers in aceto-carmine. Mitotical and meiotical aberrations and morphological variations were observed in X1 generation which were continued to X2 generation in order to stabilize the mutants.

In meiotic studies of X2, 90% of their pollen mother cells revealed the presence of an extra chromosome (Fig. 1). Observations on the morphological variations, meiotic aberrations and their subsequent behaviour indicated that all the mutants and their X2 plants carried chromosomal anomalies.

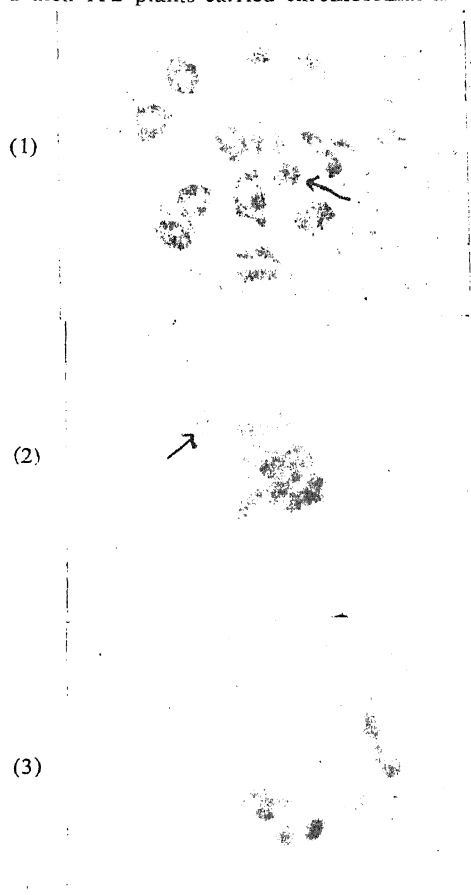


FIG. 1-3. Fig. 1. An extra chromosome at Diakinesis (arrow)  $\times$  ca. 2,000. Fig. 2. The irregular orientation of the extra chromosome at metaphase I (arrow)  $\times$  ca. 2,000. Fig. 3. Anaphase I with lagging and a bridge ( $\times$  2,000).

The most common anomaly was the formation of univalent at diakinesis and the subsequent disturbance resulting from it, such as irregular orientation at metaphase I (Fig. 2) and lagging at anaphase I (Fig. 3).

All the mutants were characterized by the increase in the size of the flower, the height and the yield. Similar characters were observed in 60% of the total number of two hundred plants. While Patil<sup>6</sup> reported adverse effects on *Arachis hypogaea*, economically useful characters such as increase in the yield and early maturity were seen in *Capsicum annum*.

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#### STOMATAL POLYMORPHISM IN *PRUNUS PADUS* L.

WHILE studying the epidermal structures in Rosaceae, the authors have observed polymorphic stomata in *Prunus padus* L. Since polymorphic stomata have not been reported so far for any member of the Rosaceae, it prompted the publication of this report.

The stomata were studied from the mature leaves obtained from the herbarium specimens of *Prunus padus* collected from Siklis in Nepal. The leaves are hypostomatic with ranunculaceous type of stomata. The stomata were measured at random from different regions of the same leaf and when the measurements (length/breadth) were plotted on a

graph, the stomata fell in three distinct groups (Fig. 1). These three groups of stomata may also

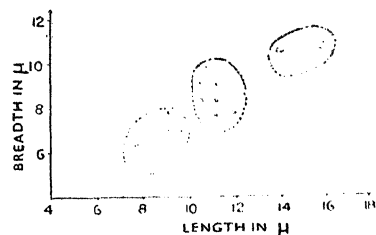


Fig. 1.

be distinguished on the basis of striations pattern. In the largest stomata, the striae extend in all directions with the radiating centres at frequent intervals around the guard cells. The striae arise in two lateral groups in the stomata of medium size. The centre of radiation of striae is not traceable in the smallest stomata and they pass parallel to the guard cells. The average number of stomata per sq. mm is 604 taken all the three types together. Out of these 53.1% are the smallest; 30.4% of the middle size and 16.5% of the largest size. The means and standard deviations of the three sizes of stomata is given in Table I.

TABLE I

|         | Size of stomata in μ |           |           |
|---------|----------------------|-----------|-----------|
|         | Small                | Medium    | Large     |
| Length  | 8.5 ± .6             | 11.4 ± .5 | 15.1 ± .7 |
| Breadth | 6.4 ± .6             | 8.6 ± .9  | 10.5 ± .3 |

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#### OXYGEN CONSUMPTION AND METABOLIC RATE IN RELATION TO BODY SIZE IN *MARTESIA STRIATA* (LINN.)

STUDIES on the oxygen consumption of marine wood boring organisms are very few<sup>1,4</sup>. Eltringham<sup>4</sup> recorded consumption of oxygen in relation to salinity in a crustacean wood borer *Limnoria*. Lane and Co-workers<sup>1,2</sup> examined the oxygen consumption of both adult and larval forms of molluscan wood borer *Teredo*. In India, respiratory studies on the pholad wood borer *Martesia fragilis* in relation to body size have been undertaken from Madras harbour<sup>3</sup>. Water filtration rate of another pholad *M. striata*, of Visakhapatnam, has been studied by Nagabhushanam<sup>5</sup>, but the relationship between body size and oxygen consumption of this local species is not known. In the present investigation, work was therefore initiated to examine the respiration of *M. striata* (Linn.) and to determine the metabolic rate in relation to body size.

Animals of different sizes were removed from the timber exposed at Visakhapatnam harbour without damaging their shell and the experiments were conducted at temperature  $25^{\circ} \pm 0.5^{\circ} \text{C}^{\circ}$ . Animals (0.077 g to 1.02 g) were taken in individual respiratory chambers and the total oxygen consumed was determined at the end of four hours.

The relationship between the body size and oxygen uptake has been obtained from the following equation<sup>7</sup>:

$$Y = a.X^b$$

where  $Y$  = oxygen consumption in ml/hr,  $X$  = the body weight, while  $a$  &  $b$  are constants.

The value of  $b$  in the above equation was determined to be 0.5665 and is thus nearer to the two-thirds power of body weight. A regression curve of oxygen consumption to body size (Fig. 1)

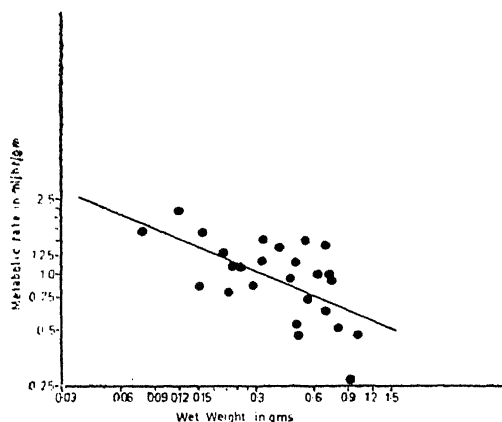


FIG. 1. Double log plot of oxygen consumption vs body weight in *Martesia striata*.

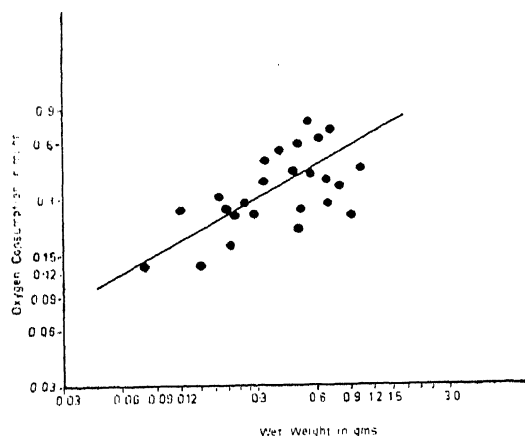


FIG. 2. Double log plot of metabolic rate against body weight.

shows that the oxygen consumption of animal increases with an increase in the size. The oxygen

consumption varied from 0.131 to 0.7880 ml/hr in the various size groups examined. The oxygen consumption per unit weight or metabolic rate varied from 0.2664 to 2.202 ml/gm/hr being inversely proportional to the body size (Fig. 2).

Although oxygen consumption in *M. striata* shows an exponential relationship to body size, in the same size group itself great variations were evident (Fig. 1). Ghiretti<sup>8</sup> observed that metabolic rate varied widely within a single species as a result of both intrinsic and extrinsic factors in molluscs. Further even under constant external conditions, the oxygen consumption of a given specimen is extremely variable<sup>8</sup>. The variations recorded in the experiments therefore do not show tendencies unusual to bivalves. The value of  $b$  observed in the present studies was also found to be very close to the value obtained for an allied wood borer *M. fragilis* earlier<sup>3</sup>.

The authors are grateful to Captain P. R. Sen, IN., Director, and Sri. S. V. S. Rao, Deputy Director of this laboratory, for their encouragement to this investigation.

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April 5, 1974.

N. KALYANASUNDARAM.

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#### CROSSING TECHNIQUE IN MUNG BEAN (*PHASEOLUS AUREUS* ROXB.)

ARTIFICIAL crossing in *Phaseolus* species is considered to be a difficult proposition, primarily due to high percentage of flower drop. Coupled with this problem is the lack of precise information about the technique of crossing. Boiling *et al* (1961) have described the method of emasculation and pollination but have not given any information about the appropriate time of emasculation and pollination. A preliminary investigation was, therefore, undertaken to determine the optimum time

TABLE I  
Average pod setting in *Phaseolus mungo* after different times of  
emasculation and pollination

| Emasculation timing |                    | Pod set per plant (no.) |      |       |       |         |      |       |       |
|---------------------|--------------------|-------------------------|------|-------|-------|---------|------|-------|-------|
|                     |                    | Morning                 |      |       |       | Evening |      |       |       |
|                     |                    | Close                   | Open | Dropt | Total | Close   | Open | Dropt | Total |
| Morning             | Flower emasculated | —                       | 51   | —     | 51    | 5       | 4    | —     | 86    |
|                     | Pod set            | —                       | —    | —     | —     | 0       | 11   | 0     | 11    |
| Evening             | Flower emasculated | 1                       | 74   | 17    | 102   | —       | 90   | —     | 90    |
|                     | Pod set            | 0                       | 17   | 0     | 17    | —       | —    | —     | —     |

of emasculation, pollination and the selection of proper bud stage.

This investigation was confined to early, semi-early and developed stages of immature mung. ME1 and ME4 (ME1 was used as female parent and ME4 as male parent). Two different times of emasculation and pollination, i.e. morning from 8.00 to 11.00 a.m. and evening from 4.00 to 6.30 p.m. were chosen. The results in four combinations, namely (i) morning emasculation, morning pollination; (ii) morning emasculation, evening pollination; (iii) evening emasculation, evening pollination and (iv) evening emasculation followed by pollination next morning. Buds of three different stages recognized by the colour, i.e. green, yellowish green, and greenish yellow, were emasculated. The emasculation and pollination of flower buds were done as suggested by Hoeling *et al.* (1961). At the time of pollination the emasculated buds were observed to be closed, opened and dropped. It was noted that emasculated buds of green colour either dropped before pollination or during pollination. Yellowish green emasculated buds were fully blossomed (open stage) while greenish yellow emasculated buds were observed to be closed at the time of pollination. Therefore, only the last two categories of buds were pollinated.

The emasculated buds were pollinated according to the schedule. The data regarding the flowers emasculated and the pod setting in different flower stages are presented in Table I.

It is clear from Table I that evening emasculation followed by next morning pollination gave the highest pod setting of 17 pods out of 102 buds emasculated (17%). and the next in order was the morning emasculation followed by evening pollination resulting in 11 pods out of 86 buds emasculated (13%). Based on number of pollinations made the per cent pod setting was 20% and 14% respectively. Furthermore, it was noted that simultaneous emasculation and pollination either in the morning or evening gave very low pod set, viz. 5.6%.

It was very much interesting to note that the flowers that remained closed at pollination time did not set any pod while whole of the pod setting was obtained in those buds which blossomed (buds) opened at the pollination time. Non setting of pods in the flowers that were closed at pollination time may be due to the fact that the female was not more receptive.

It is therefore suggested from the present study that high percentage of pod setting in *Phaseolus mungo* can be obtained by following emasculation of yellowish green (opened) stage buds in the evening (4.00 p.m. to 6.30 p.m.) and pollination of only blossomed flowers in the next morning (8.00 a.m. to 11.00 a.m.). The applicability of these findings was also tested in interspecific hybridization between *Phaseolus mungo* and *Phaseolus mungo* and a pod setting up to 34% has been achieved.

Dept. of Plant Breeding, I. P. Singh  
Punjab Agricultural Univ., R. S. MALHOTRA  
Ludhiana, January 1, 1974

L. Hoeling, M. Sander, D. A. and Matlock, R. S.  
*Genom. J.* 1961, 33, 54

## ONTOGENY, STRUCTURE AND DISTRIBUTION OF TRICHOMES ON THE FLORAL PARTS OF *CITRUS COROMANDELICA* VAHL.

Various types of trichome occur on plants and their taxonomic significance has been emphasized by several workers, especially in respect of the Umbelliferae (Compositae) (Carlquist<sup>1,2</sup>, Ramayya<sup>3</sup>), Labiales (Heintzelman and Howard<sup>4</sup>), Labiate (Mathias<sup>5</sup>), Gentianaceae (Laroche and Siddiqui<sup>6</sup>) and Scrophulariaceae (Kaur<sup>7</sup>). Bachmann<sup>8</sup> investigated the structure of hairs in many angiosperm taxa and presented a key for their identification.

The present note deals with the ontogeny, structure and distribution of trichomes on the floral parts of *Citrus coromandeliana*. The buds and flowers were collected from the bank of river

Animals of different sizes were removed from the timber exposed at Visakhapatnam harbour without damaging their shell and the experiments were conducted at temperature  $25^{\circ} \pm 0.5^{\circ} \text{C}^{\circ}$ . Animals (0.077 g to 1.02 g) were taken in individual respiratory chambers and the total oxygen consumed was determined at the end of four hours.

The relationship between the body size and oxygen uptake has been obtained from the following equation<sup>7</sup>:

$$Y = a \cdot X^b$$

where  $Y$  = oxygen consumption in ml/hr.  $X$  = the body weight, while  $a$  &  $b$  are constants.

The value of  $b$  in the above equation was determined to be 0.5665 and is thus nearer to the two-thirds power of body weight. A regression curve of oxygen consumption to body size (Fig. 1)

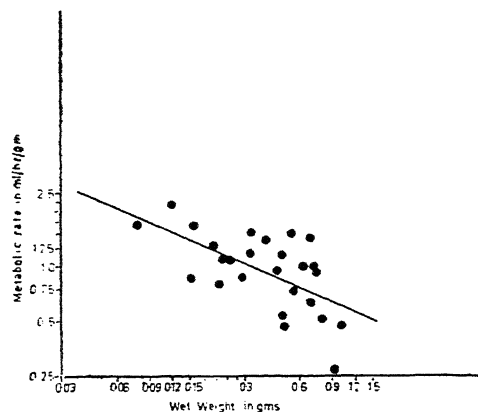


FIG. 1. Double log plot of oxygen consumption vs body weight in *Martesia striata*.

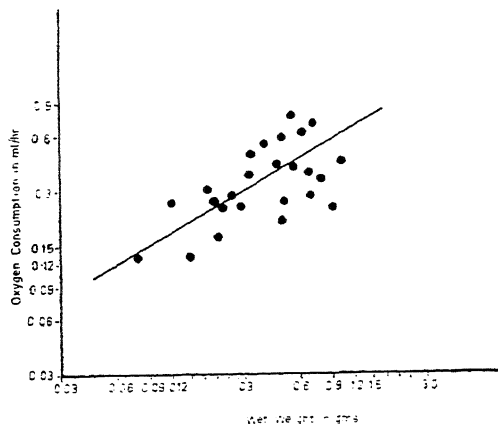


FIG. 2. Double log plot of metabolic rate against body weight.

shows that the oxygen consumption of animal increases with an increase in the size. The oxygen

consumption varied from 0.131 to 0.7880 ml/hr in the various size groups examined. The oxygen consumption per unit weight or metabolic rate varied from 0.2664 to 2.202 ml/gm/hr being inversely proportional to the body size (Fig. 2).

Although oxygen consumption in *M. striata* shows an exponential relationship to body size, in the same size group itself great variations were evident (Fig. 1). Ghirelli<sup>8</sup> observed that metabolic rate varied widely within a single species as a result of both intrinsic and extrinsic factors in molluscs. Further even under constant external conditions, the oxygen consumption of a given specimen is extremely variable<sup>8</sup>. The variations recorded in the experiments therefore do not show tendencies unusual to bivalves. The value of  $b$  observed in the present studies was also found to be very close to the value obtained for an allied wood borer *M. fragilis* earlier<sup>8</sup>.

The authors are grateful to Captain P. R. Sen, IN., Director, and Sri. S. V. S. Rao, Deputy Director of this laboratory, for their encouragement to this investigation.

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April 5, 1974.

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#### CROSSING TECHNIQUE IN MUNG BEAN (*PHASEOLUS AUREUS* ROXB.)

ARTIFICIAL crossing in *Phaseolus* species is considered to be a difficult proposition, primarily due to high percentage of flower drop. Coupled with this problem is the lack of precise information about the technique of crossing. Boiling *et al* (1961) have described the method of emasculation and pollination but have not given any information about the appropriate time of emasculation and pollination. A preliminary investigation was, therefore, undertaken to determine the optimum time

TABLE I  
Number of flowers emasculated and pod set in different stages

| Emasculation<br>timings |                     | Pollination timings           |      |      |       |         |      |      |       |
|-------------------------|---------------------|-------------------------------|------|------|-------|---------|------|------|-------|
|                         |                     | Morning                       |      |      |       | Evening |      |      |       |
|                         |                     | Bud stage at pollination time |      |      |       |         |      |      |       |
|                         |                     | Close                         | Open | Drop | Total | Close   | Open | Drop | Total |
| Morning                 | Flowers emasculated | ..                            | 81   | ..   | 81    | 33      | 47   | 6    | 86    |
|                         | Pod set             | ..                            | 5    | ..   | 5     | 0       | 11   | 0    | 11    |
| Evening                 | Flowers emasculated | 31                            | 54   | 17   | 102   | ..      | 90   | ..   | 90    |
|                         | Pod set             | 0                             | 17   | 0    | 17    | ..      | 5    | ..   | 5     |

of emasculatation, pollination and the selection of proper bud stage.

This investigation was conducted using two newly developed strains of mungbean, namely, ML1 and ML4. ML1 was used as female parent and ML4 as male parent. Two different times of emasculatation and pollination, viz., morning from 8-00 to 11-00 a.m. and evening from 4-00 to 6-30 p.m. : were chosen. This resulted in four combinations, namely : (i) morning emasculatation, morning pollination, (ii) morning emasculatation, evening pollination, (iii) evening emasculatation, evening pollination and (iv) evening emasculatation followed by pollination next morning. Buds of three different stages recognised by the colour, viz., green, yellowish green, and greenish yellow were emasculated. The emasculatation and pollination of flower buds were done as suggested by Boiling *et al.* (1961). At the time of pollination the emasculated buds were observed to be closed, opened and dropped. It was noted that emasculated buds of green colour either dropped before pollination or during pollination. Yellowish green emasculated buds were fully blossomed (open stage), while greenish yellow emasculated buds were observed to be closed at the time of pollination. Therefore, only the last two categories of buds were pollinated.

The emasculated buds were pollinated according to the schedule. The data regarding the flowers emasculated and the pod setting in different flower stages are presented in Table I.

It is clear from Table I that evening emasculatation followed by next morning pollination gave the highest pod setting of 17 pods out of 102 buds emasculated (17%) and the next in order was the morning emasculatation followed by evening pollination resulting in 11 pods out of 86 buds emasculated (13%). Based on number of pollinations made the per cent pod setting was 20% and 14% respectively. Furthermore, it was noted that simultaneous emasculatation and pollination either in the morning or evening gave very low pod set, viz., 5-6%.

It was very much interesting to note that the flowers that remained closed at pollination time did not set any pod, while whole of the pod setting was observed in those buds which blossomed (remained open) at the pollination time. Non-setting of pods in the flowers that were closed at pollination time may be due to the fact that the female was no more receptive.

It is, therefore, suggested from the present study that high percentage of pod setting in *Phaseolus aureus* can be obtained by following emasculatation of yellowish green (open) stage) buds in the evening (4.00 p.m. to 6.30 p.m.) and pollination of only blossomed flowers in the next morning (8-00 a.m. to 11.00 a.m.). The applicability of these findings were also tested in interspecific hybridization between *Phaseolus aureus* and *Phaseolus mungo* and a pod setting up to 34% has been achieved.

Depr. of Plant Breeding,  
Punjab Agricultural Univ.,  
Ludhiana, January 1, 1974.

T. P. SINGH.  
R. S. MALHOTRA.

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# ONTOGENY, STRUCTURE AND DISTRIBUTION OF TRICHOMES ON THE FLORAL PARTS OF *CELSIA COROMANDELIANA* VAHL.

VARIOUS types of trichome occur on plants and their taxonomic significance has been emphasized by several workers especially in respect of the families Compositae (Carlquist<sup>2-4</sup>, Ramayya<sup>9</sup>); Icacinaceae (Heintzelman and Howard<sup>6</sup>); Labiatae (Mathur<sup>8</sup>); Lentibulariaceae (Farooq and Siddiqui<sup>5</sup>) and Scrophulariaceae (Kaur<sup>7</sup>). Bachmann<sup>1</sup> investigated the structure of hairs in many angiosperm taxa and presented a key for their identification.

The present note deals with the ontogeny, structure and distribution of trichomes on the floral parts of *Celsia coromandeliana*. The buds and flowers were collected from the bank of river



Yamuna, Delhi, and were fixed in formalin-acetic-alcohol. Conventional methods of dehydration, infiltration and embedding were followed. Sections were cut at a thickness of 5–8 microns and stained either in safranin or iron-alum haematoxylin with fast green as counterstain.

**Trichomes on Bracts and Sepals.**—The trichome is initiated as a protuberance from any epidermal cell which is distinguishable by its dense cytoplasm and conspicuous nucleus (Fig. 1A). This cell elongates and divides transversely to form a terminal and a basal cell (Fig. 1B). Further divisions are confined to the terminal cell whereas the basal cell

functions as the foot (Fig. 1B–F). The derivatives of the terminal cell lead to the formation of a file of two (Fig. 1C), three (Fig. 1D) and four cells (Fig. 1E). Further divisions in the apical cell of the file, however, are vertical and result in the formation of head (Fig. 1F–H). In its final structure the multicellular trichome can be divided into three regions: (a) Foot, (b) Stalk, made up of 3 or 4 septate, highly vacuolated cells, with meagre cytoplasm and (c) Head, comprising 4 to 8 cells with dense cytoplasm and prominent nuclei. Trichomes of this type occur abundantly on bracts and sepals, are absent from the petals and sporadically occur on the pistil.

**Trichomes on Stamens.**—The trichomes on the basal part of the filament are long, unicellular and non-septate. An epidermal cell destined to develop as a trichome elongates to form a long, unicellular structure (Fig. 1I–M). The nucleus, which is initially situated at the base (Fig. 1J), migrates towards the tip as the hair matures (Fig. 1M).

Studies on the ontogeny, structure and distribution of the epidermal appendages of the floral parts of *Verbascum thapsus* (unpublished observations) show that they are characterised by the presence of highly branched trichomes on the bracts and sepals. The pistil is also heavily clothed with numerous branched hairs. *Celsia coromandeliana* differs from *Verbascum thapsus* in having uniseriate trichomes on bracts and sepals and very few trichomes on the pistil. The present study indicates that *C. coromandeliana* is not *pro parte V. thapsus* and supports their status as independent taxa.

It gives me great pleasure to express my gratitude to Dr. M. R. Vijayaraghavan for encouragement and suggestions.

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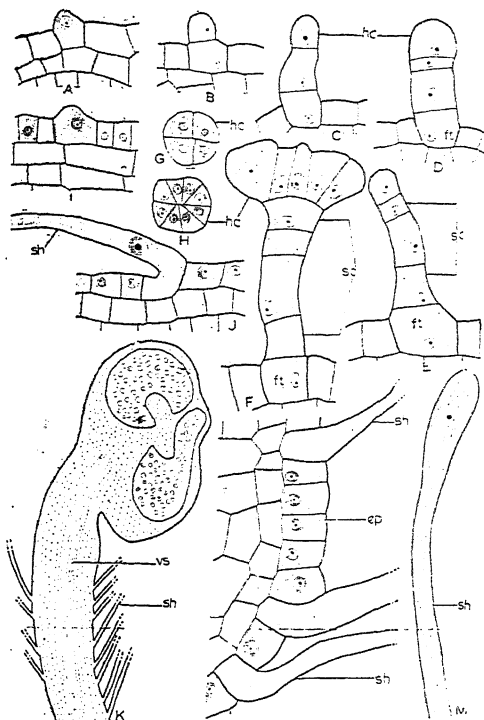


FIG. 1. A–M. Ontogeny of trichomes in *Celsia coromandeliana*. A. Hair initial.  $\times 342$ ; B–E. Two, three, four and five-celled stages of trichomes respectively,  $\times 342$ ; F. Trichome with a head,  $\times 342$ ; G, H. Transections through four- and eight-celled head of trichome,  $\times 342$ ; I, J. Portions of staminal filaments showing elongation of epidermal cells to form unicellular hairs,  $\times 342$ ; K. Longitudinal section of stamen (diagrammatic) to show the distribution of trichomes on the filament,  $\times 342$ ; L. Portion of filament to show details of the basal part of the trichomes,  $\times 342$ ; M. Mature hair showing a terminally situated nucleus,  $\times 142$ . (ep, epidermis; ft, foot cell; hc, head cell; sc, stalk cells; sh, staminal hair; vs, vascular strand.)

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## SHORT SCIENTIFIC NOTES

### Phytochemical Studies on *Cassia* Species of Indian Arid Zone

Sennosides are the active principles of senna and are well known for their medicinal importance<sup>3,4</sup>. Although sennoside percentage of Tinnevely senna (*C. angustifolia*) and Alexandrian senna (*C. acutifolia*) has been estimated earlier, so far no such work has been done on *Cassia* species growing in Indian arid zone<sup>1,2</sup>. During the present investigation an attempt has been made to estimate the

in the leaves and seeds of wild *C. angustifolia* have been reported to be 3.0 to 5.0 and 2.4 to 3.0% respectively<sup>6,7</sup>.

Grateful thanks of the authors are due to ICAR, New Delhi, for the scheme sanctioned to one of us (DDC), which made this study possible. They are also thankful to Professors H. C. Arya and R. C. Kapoor for the facilities provided and to Dr. D. N. Sen, Reader in Botany, for guidance and critically reading the manuscript.

TABLE I  
Percentage of sennoside contents in the leaf samples of *Cassia* species

| Plant species          | Time of collection, stage and place                               | Ratio $E_{515}/E_{440}$ | Total sennoside % | Rhein carboxylic derivatives |         |
|------------------------|---|-------------------------|-------------------|------------------------------|---------|
|                        |   |                         |                   | Ratio $E_{515}/E_{440}$      | Rhein % |
| <i>C. angustifolia</i> | Three months old, flowering, botanical garden, Jodhpur University | 1.41                    | 4.23              | 1.40                         | 3.54    |
| <i>C. fistula</i>      | Three, flowering April 1974, botanical garden, Jodhpur University | 1.26                    | 1.80              | 1.33                         | 1.23    |
| <i>C. javanica</i>     | Tree, flowering April 1974, Mandore garden, Jodhpur               | .65                     | 0.20              | ..                           | ..      |
| <i>C. siamea</i>       | do.   | 0.30                    | 0.07              | 0.75                         | 0.05    |
| <i>C. tora</i>         | Two months old, vegetative, botanical garden, Jodhpur University  | 0.60                    | 0.14              | 0.70                         | 0.11    |
| <i>C. sophera</i>      | do.   | 1.25                    | 0.07              | 0.75                         | 0.05    |
| <i>C. auriculata</i>   | Shrub, fruiting, December 1973 Somesar (Pali District, Rajasthan) | 0.38                    | 0.15              | 0.33                         | 0.77    |

sennoside contents in the leaves of various *Cassia* species from Western Rajasthan.

For this study, the leaves samples were first air dried at room temperature. All the measurements were recorded on Spectronic-20 spectrophotometer, and sennoside estimation was done as reported in B.P.C. (British Pharmacopoeia)<sup>2,5</sup>. Besides the total sennosides content in *Cassia* leaves the rhein carboxylic derivatives, equivalent to sennoside B, have also been estimated. The experimental observations for plant materials (leaves) and their sennoside contents has been presented in Table I.

It appeared from the present studies that *C. angustifolia* contained highest sennoside contents followed by *C. fistula*. It was interesting to note that besides sennosides, the percentage of rhein was also maximum in *C. angustifolia* when compared with other *Cassia* species. The sennoside contents

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**Fruit Rot of *Trichosanthes dioica* L. Caused by *Pythium cucurbitacearum* Takimoto in West Bengal**

Plants belonging to the family Cucurbitaceae are susceptible to a few *Pythium* spp. Commonest infections are those caused by *P. aphanidermatum* (Ed.) Fitz<sup>1</sup>. Takimoto (1941) from Japan first reported *P. cucurbitacearum* sp. nov. on a few cucurbitaceous plants. Samples of rotten fruits of *Trichosanthes dioica* collected from fields of Nadia District, West Bengal, has yielded *P. cucurbitacearum* Tak.

Infections generally start from the styler end and characteristically cause extensive soft rotting with fine mycelial growth on the surface at later stages. Diagnostic characters of the species as noted on host are as follows:

Hyphae broad, 4–6  $\mu$  in breadth; zoosporangia papillate, terminal, on short sporangiophores, mostly spherical, varying to ovoid, thin-walled with granular protoplast, measuring 22–35  $\mu \times$  20–30  $\mu$ ; oogonia, on long slender hyphae, are spherical containing mostly central, rarely peripheral oosphere, measuring respectively, 20–25  $\mu$  and 12–16  $\mu$  in diameter. Antheridia (lobes) nearly spherical to clavate, making basal contact with oogonial lobe at the point of its origin, may be rarely bilobed, measuring 8–10  $\mu$  in diameter. Copulation amphigynous, antheridial nucleus passing apparently through a pore at contact. Oospores spherical, thick-walled, uniform, measuring 15–18  $\mu$ .

The species *P. cucurbitacearum* Tak. is a new record for India and *Trichosanthes dioica* is an addition to the list of its hosts. Material is preserved in the Herbarium, Department of Plant Pathology, Kalyani University.

Dept. of Plant Pathology. S. CHAUDHURI.  
University of Kalyani,  
West Bengal, July 29, 1974.

1. Butler, E. J. and Bisby, G. R., *The Fungi of India*, revised by R. S. Vasudeva, ICAR, New Delhi, 1960, p. 552.
2. Takimoto, S., "On the *Pythium* causing damping off of seedling and fruit rot of cucumber," *Ann. Phytopath. Soc. Japan*, 1941, 11, 89.

**Morphogenesis in Seed Cultures of *Spathoglottis***

Orchids produce millions of seeds per capsule, but only a few germinate and attain blooming. The orchid breeders generally follow vegetative propagation. For over 50 years successful attempts have been made to germinate orchid seeds on agar media<sup>1,2</sup>. This communication describes the germination of seeds, and morphogenesis of embryo in *Spathoglottis plicata* in vitro.

For the present investigation, the plants with violet flowers were selected. The seeds were asepti-

cally scooped from mature capsules, and were placed on a modified White's agar medium with 2% sucrose (BM), and also on BM supplemented with casein hydrolysate (CH), coconut milk (CM), 2, 4-D, IAA, NAA, and kinetin, individually and in different combinations. Each culture received about 40 seeds, and cultures were maintained under controlled conditions of light (8–10 ft candles), temperature (25°C  $\pm$  2) and humidity (about 60%).

The seeds at culture contained an undifferentiated embryo enclosed by a thin, transparent seed coat. In the majority of the cultures, they swelled within 2–3 weeks. On BM about 45% seeds germinated. On the contrary, a significant response was noted on BM + CM (10%) and BM + CH (1000 ppm) with 60% and 80% germination, respectively, and satisfactory growth of seedlings. But, on BM + CM (10%) + CH (1000 ppm) + IAA (1 ppm) + kinetin (1 ppm) and BM + CH (1000 ppm) + IAA (1 ppm) + 2, 4-D (ppm) only 25% and 20%, respectively, produced seedlings. During germination, the swollen embryo emerged from the seed coat as a creamy-white, or greenish, spherule which later developed into a 'top'-shaped protocorm with numerous unicellular rhizoids. Within 6–7 weeks of culture, from the protocorm differentiated a shoot apex which developed into a shoot with 2 or 4 leaves; and 2 or 3 roots in another 2 weeks. By about the 13th week we obtained a fully-differentiated plant with 6–8 leaves and 4–6 roots.

In addition, the seeds also exhibited several interesting features like callusing, abnormal seedlings with thick, negatively-geotropic roots, and differentiation of adventitious buds on protocorms. The buds further developed into multiple shoots. The callus was subculturable, and rapid growth occurred on BM + 2,4-D (0.5 and 1 ppm) + NAA (0.5 to 2 ppm). When subcultured on BM, 8% cultures differentiated roots, whereas on BM + CH (1000 ppm) 90% cultures developed vigorously growing roots.

The hypocotyl segments showed a high degree for differentiation of roots and shoots resulting in plantlets. The latter were transferred to pots containing vermiculite and, subsequently, soil. In the green house the plantlets developed several new leaves and reached a height of 15–20 cm in about 4 weeks.

Department of Botany. M. S. CHENNAVEERIAH.  
Karnatak University. SAROJINI J. PATIL.  
Dharwar 580003, Karnataka,  
July 17, 1974.

1. Arditti, J., *Bot. Rev.*, 1967, 33, 1.
2. Steward, F. C. and Mapes, M. O., *Bot. Gaz.*, 1971, 132, 65.

# Occurrence of Giant Potholes in the Deccan Trap Lava Flows

Formation of potholes though quite characteristic of the sedimentary rock formations, particularly those of the limestone formations in many parts of the country, they are not so common in the Deccan Trap lava flows. The Kukari river, a tributary to Ghod river in Bhima basin, has given rise to some excellent potholes near village Nighoj (Toposheet No. 47 J/5. Quadrant 1 A, 18° 56' : 74° 16' R.L. 588 metres above m.s.l.). The river has a perennial flow at this place but the stream has water falls (45 m deep) at the location of the potholes. The potholes exist only locally for about half a kilometer in the direction of the stream, with cascade flow due to ungraded slope of the stream bed. The diameter of the giant potholes vary from 1 meter to 3 meters, the depth being about 80 meters. In the advanced stages, the potholes are interconnected along the side walls.

The bedrock of the lava flow is quite hard and compact nevertheless the stream has chiselled out the huge bowels. The following sequence of the lava flows could be demarcated :

I. Top-flow : Greyish, hard and compact, well jointed, highly siliceous, porphyritic basalt. Nodules of cryptocrystalline silica are abundant. Thin veins of brecciated basalt about 10 cm in width criss-cross this flow.

II. Second-flow : Greyish black, poorly jointed hard and compact fine grained basalt.

The agate-rich surface lava flow with pentagonal joints shows circular rings of iron bands inside the pentagons suggesting the potential nuclei of future potholes.

Deputy Director,  
Groundwater Surveys and  
Development Agency,  
Government of Maharashtra,  
Pune-9. August 1, 1974.

R. N. RAKSHIT.

## Books Received

*Annual Review of Genetics* (Vol. 7). Edited by H. L. Roman, L. M. Sandler and A. Campbell. (Annual Reviews, Inc., Palo Alto, California 94306, U.S.A.), 1973. Pp. vii + 504. Price : U.S.A. \$ 12.00 ; Foreign \$ 12.50.

*Annual Review of Entomology* (Vol. 19). By R. F. Smith, T. E. Mittler and C. N. Smith. (Annual Reviews, Inc., 4139, El Camino Way, Palo Alto, California 94306), 1974. Pp. vii + 512. Price \$ 12.00 U.S.A. ; Elsewhere \$ 12.50.

*Fundamentals of Nuclear Science (with Application in Agriculture and Biology)*. By P. N. Tiwari. Wiley Eastern Private Ltd., J 41, South Extension 1, New Delhi, 110049), 1974. Pp. xi + 167. Price Rs. 26.00.

*Tables of Physical and Chemical Constants and Some Mathematical Functions*. By G. W. C. Kaye and T. H. Laby. (Longman Group Ltd., London). Pp. xi + 386. Price £ 3.95.

*The Protein in Chemistry* (2nd Edition). By R. P. Bell. (Chapman and Hall Ltd., 11 New Fetter Lane, London EC 4, P4 EE). 1974. Pp. vii + 310. Price £ 6.30.

*Annual Review of Physiology* (Vol. 36). 1974, (Annual Reviews, Inc., Palo Alto, California 94306). Price : USA, \$ 12.00 ; elsewhere \$ 12.50.

*Design and Evaluation of Irrigation Methods*. By A. M. Michael, Shri Mohan and K. R. Swaminathan. (Water Tech. Centre, Indian Agricultural Research Institute, New Delhi 12), 1972. Pp. 208. Price Rs. 15.00.

*Water Resources of India and Their Utilisation in Agriculture*. By C. Dakshinamurti, A. M. Michael, and Shri Mohan. (Water Tech. Centre, Indian Agricultural Research Institute, New Delhi 12), 1973. Pp. xvi + 400. Price Rs. 25.50.

*Irrigation with Saline Water*. By K. V. Paliwal. (Water Tech. Centre, Indian Agricultural Research Institute, New Delhi 12), 1972. Pp. 198. Price Rs. 16.00.

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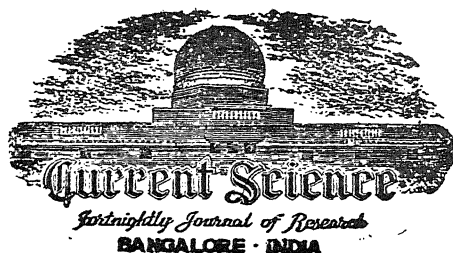
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# X-RAY INTENSITY STATISTICS OF APPROXIMATELY CENTROSYMMETRIC STRUCTURES\*

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## ABSTRACT

The joint probability distribution of X-ray structure factors of a non-centrosymmetric structure (true structure) and that of a centrosymmetric structure (assumed model) from which it deviates slightly, are given. These are characterised by a parameter  $D = \langle \cos 2\pi \mathbf{H} \cdot \Delta \mathbf{r}_j \rangle$  where  $\Delta \mathbf{r}_j$  are the deviations of the  $N$  atoms from the centrosymmetric arrangement. The limiting forms of the distribution for  $D = 0$ , and  $D = 1$  are respectively the acentric and centric distributions of Wilson. Intermediate values of  $D$  characterise different degrees of centrosymmetry of the structure. The parallelism of the results to another 'approximately centrosymmetric' situation (Srinivasan, *Indian J. Pure Appl. Phys.*, 1965, 3, 187), is pointed out.

## 1. INTRODUCTION

IN a series of papers from this laboratory (Ramachandran *et al.*, 1963; Srinivasan *et al.*, 1963 *a, b*; 1964; Srinivasan and Ramachandran, 1965 *a, b*; 1966; Srinivasan and Chandrasekharan, 1966; Parthasarathy and Srinivasan, 1967) the statistical distribution of a pair of structure factors have been considered. The results obtained therein have been shown to be applicable to different stages of crystal structure refinement as well as for testing of isomorphism between a pair of crystals. The probability distributions established were for a pair of structure factors,  $F_N$  and  $F_P^c$  where  $F_N$  corresponds to the true structure containing  $N$  atoms and  $F_P^c$  to the assumed model containing a part ( $P$  atoms) of the structure whose coordinates  $\mathbf{r}_{Pj}^c$  differ from the true  $\mathbf{r}_{Nj}^c$  by  $\Delta \mathbf{r}_{Nj}$ . Broadly, two cases have been considered so far namely (a) when both  $F_N$  and  $F_P^c$  are non-centrosymmetric (case I) and (b) when both  $F_N$  and  $F_P^c$  are centrosymmetric (case II). The various results that have been deduced are thus applicable to a crystal structure which is known to be either non-centrosymmetric or centrosymmetric as the case may be.

The possibility that  $F_P^c$  could correspond to a centrosymmetric model while  $F_N$  is non-centrosymmetric (we shall refer to this as case III) and *vice versa* (case IV) was not considered earlier since it was thought that this might not have immediate practical application. It has however been realised now that these cases are also of interest since they could lead to distributions for pseudosymmetric structures.† Consider for simplicity the case  $P = N$ .

It is possible to imagine a structure which is in reality non-centrosymmetric while the assumed model is centrosymmetric. Following the basic problem posed for cases I and II, we postulate that the  $N$  atoms of the true structure are related to the  $N$  atoms of the assumed model through the given set of errors  $\Delta \mathbf{r}_{Nj}$ . Since the assumed model is centrosymmetric for the present case, this would mean that the true model could better be described as "approximately centrosymmetric". Thus one would expect that as  $\Delta \mathbf{r}_{Nj}$  tend to zero the distribution would tend to be a centric one while when  $\Delta \mathbf{r}_{Nj}$  tend to be large the distribution would tend to the acentric one. For intermediate values of  $\Delta \mathbf{r}_{Nj}$  the distribution can be taken to represent different degrees of centrosymmetry of a non-centrosymmetric structure.

It would appear that the statistics of such a situation was first considered by Luzzati (1953) who applied his earlier analysis (1952) to the above problem and worked out theoretically the values to be expected for a type of discrepancy index involving  $F_N$  and  $F_N^c$  which would enable one to use the results to deduce  $\langle |\Delta \mathbf{r}_{Nj}| \rangle$  for a practical case. His treatment of the problem is brief and is restricted to the statement of a few results. Recently this aspect of the problem has been considered in this laboratory in detail following the type of analysis done for cases I and II. The purpose of this paper is to outline briefly some of the main steps involved in deducing the distributions and the possible application of these to develop other statistical criteria. Most of these could be deduced following the same procedures as for cases I and II and in conjunction with Luzzati's treatment (1953).

It may be mentioned that the problem of this type of degree of centrosymmetry of a non-centrosymmetric structure was also recently considered in this laboratory (Srinivasan and Vijayalakshmi, 1972 *a, b*; Srinivasan *et al.*, 1974; Srinivasan, Swaminathan and Chacko, 1972) and the present

\* Contribution No. 393 from the Centre of Advanced Study in Physics, University of Madras, Madras-600025, India.

† Following Srinivasan (1965 *a*) we use this term pseudosymmetry in a very general sense to denote the presence of any non-crystallographic symmetry property associated with the "asymmetric part" or "crystallographic unit" so as to invalidate the basic Wilson conditions.



analysis forms, in part, an extension of the above studies.

## 2. BASIC PROBABILITY DISTRIBUTIONS

Let  $r_{Nj}$  denote the true coordinates of the structure which is approximately centrosymmetric. These may be considered to have been obtained by giving random and independent displacements  $\Delta r_{Nj}$  to the coordinates of a perfectly centrosymmetric assumed model. It is assumed that the shifts  $\Delta r_{Nj}$  and  $\Delta r_{Nj'}$  for the atoms  $j$  and  $j'$  which are related by centrosymmetry are random and independent where  $n = N/2$ . We denote by  $F_N$  and  $F_N^c$  the structure factors of the true and assumed structures. It is also assumed that both the true and assumed structures satisfy the ideal Wilson conditions, namely, they contain large number of similar atoms randomly distributed in the unit cell.

The establishment of conditional distribution  $P(F_N; F_N^c)$  follows closely the steps given earlier (e.g., see Appendix 1 of Srinivasan and Chandrasekharan, 1966) and is not detailed here.† The conditional distribution  $P(F_N, F_N^c)$  takes the form

$$P(F_N; F_N^c) = \frac{1}{\pi \sigma_N^2 (1 - D^2)} \exp \left[ - \frac{F_N^2 - D^2 (F_N^c)^2 - 2D F_N F_N^c \cos \alpha}{\sigma_N^2 (1 - D^2)} \right] \quad (1)$$

where  $D = (\cos 2\pi H \cdot r_{Nj})$ ,  $\sigma_N^2 = \sum_{j=1}^N f_j^2$

and  $\alpha$  is the angle between  $F_N$  and  $F_N^c$ . The joint distribution  $P(F_N, \alpha; F_N^c)$  for a given  $F_N^c$  is

$$P(F_N, \alpha; F_N^c) = \frac{|F_N|}{\pi \sigma_N^2 (1 - D^2)} \exp \left[ - \frac{F_N^2 - D^2 (F_N^c)^2 - 2D F_N F_N^c \cos \alpha}{\sigma_N^2 (1 - D^2)} \right] \quad (2)$$

where  $\alpha = \alpha_N - \alpha_N^c$ . In terms of normalised variables  $y_N = F_N / \sigma_N$ ,  $y_N^c = F_N^c / \sigma_N$  we have

$$P(y_N, \alpha; y_N^c) = \frac{y_N}{(1 - D^2)} \exp \left[ - \frac{y_N^2 - D^2 (y_N^c)^2 - 2D y_N y_N^c \cos \alpha}{(1 - D^2)} \right] \quad (3)$$

$$P(y_N; y_N^c) = \frac{2y_N}{(1 - D^2)} \exp \left[ - \frac{y_N^2 - D^2 (y_N^c)^2}{1 - D^2} \right] I_0 \left[ \frac{2D y_N y_N^c}{1 - D^2} \right] \quad (4)$$

† The number of atoms in the model and true structure are denoted here by  $N$  while in the paper cited (Srinivasan and Chandrasekharan, 1966) it was denoted by  $P$ . Also the only other difference is that  $F_N^c$  now corresponds to a centrosymmetric model.

It is important to note that while  $F_N$  has both amplitude and phase,  $F_N^c$  is real since it corresponds to a centrosymmetric model. Thus assuming a centric distribution for  $P(y_N^c)$

$$P(y_N^c) = \sqrt{2\pi} \exp \left[ - (y_N^c)^2 / 2 \right] \quad (5)$$

the joint distribution  $P(y_N, y_N^c)$  is readily deduced to be

$$P(y_N, y_N^c) = \sqrt{2\pi} \frac{2y_N}{(1 - D^2)} \exp \left[ - \frac{2y_N^2 - (1 - D^2)(y_N^c)^2}{2(1 - D^2)} \right] I_0 \left[ \frac{2D y_N y_N^c}{(1 - D^2)} \right] \quad (6)$$

The distribution of  $\alpha$  for a given  $F_N$ ,  $F_N^c$  will be useful. This is given by

$$P(\alpha; F_N, F_N^c) = \frac{1}{2\pi} \frac{\exp \left[ - \frac{2D F_N F_N^c \cos \alpha}{\sigma_N^2 (1 - D^2)} \right]}{I_0 \left[ \frac{2D F_N F_N^c}{\sigma_N^2 (1 - D^2)} \right]} \quad (7)$$

So also, the actual distribution of phases,  $\alpha$  can be deduced from (3) by substituting for  $P(y_N^c)$  corresponding to centric distribution and integrating over  $y_N$  and  $y_N^c$ . The expression is not given here.

## 3. DISCUSSION OF THE RESULTS

The above results form the basis for working out a number of statistical results of interest. For instance the availability of the joint distributions  $P(y_N, y_N^c)$  enables us to study distributions of variables such as the difference  $y_d = (y_N - y_N^c)$  (and also product, quotient, etc.). These will be considered in detail in a later paper. However, we shall deduce here one interesting result concerning the distribution  $P(y_N)$  alone. Thus integration over  $y_N^c$  in (6) leads to the marginal distribution  $P(y_N)$  to be

$$P(y_N) = \frac{2y_N}{\sqrt{1 - D^2}} \exp \left[ - (y_N^2 / (1 - D^2)) \right] I_0 \left[ \frac{D^2 y_N^2}{1 - D^2} \right] \quad (8)$$

This may be compared with the probability distribution of the normalised amplitude for another situation of "an approximately centrosymmetric structure" considered earlier in the literature (Srinivasan, 1965 *a, b*). This deals with the case of a non-centrosymmetric structure (space group  $P1$ ) containing a centrosymmetric and a non-centrosymmetric group. The distribution for such a case turns out to be‡

$$P(y_N) = \frac{2y_N}{\sqrt{1 - \sigma_1^4}} \exp \left[ - (y_N^2 / (1 - \sigma_1^4)) \right] I_0 \left[ \frac{\sigma_1^2 y_N^2}{1 - \sigma_1^4} \right] \quad (9)$$

‡ Although Srinivasan (1965 *a*) gave for this case  $P(y_N)$  as an integral his expression can be reduced using standard integral tables (Gradshteyn *et al.*, 1965) to the above form.

where  $\sigma_1^2$  stands for the ratio of the mean square amplitudes corresponding to the centric group to that of the entire structure. A comparison of (8) with (9) shows that the final distributions are identical in form except for the appearance of  $D$  in (8) in place of  $\sigma_1$  in (9). The parallel roles of  $\sigma_1$  and  $D$  are now obvious. Thus the two limits  $D=0$  (or  $\sigma_1=0$ ) and  $D=1$  (or  $\sigma_1=1$ ) correspond to acentric and centric distributions. Intermediate values of  $D$  (or  $\sigma_1$ ) correspond to different degrees of centrosymmetry. Thus  $D$  (or  $\sigma_1$ ) may be taken as a quantitative measure of the degree of centrosymmetry of the structure. In fact it may be pointed out that Srinivasan *et al.* (1972) suggested earlier the use of  $D$  for this purpose and the present results only supply a sound theoretical justification for the same. The curves of  $P(y_N)$  given as a function of  $\sigma_1^2$  in the earlier paper (Srinivasan, 1965 *a, b*) are applicable here.

It may be mentioned that  $D$  can be estimated in practice by working out some of the statistical parameters connected with  $y_N$ . For instance, the moments or variance of  $y_N$  can be worked out which turn out to be functions of  $D$ . However, these may not be very sensitive especially when  $\Delta r_{Nj}$  are small. It seems preferable to turn to other statistical parameters involving quantities  $y_N$  and  $y_N^c$  such as say the difference  $y_d = y_N - y_N^c$ . Since it measures the deviation of  $y_N$  from that of the assumed model, this and other related parameters such as reliability indices based on it may be expected to be more sensitive.

We may also mention here that since  $y_N$  and  $y_N^c$  now involve non-centrosymmetric and centrosymmetric combination, distributions connected with pair such as say the quotients  $y_N/y_N^c$ ,  $y_N^c/y_N$  need

not show symmetry properties such as were associated with these variables for cases I and II. These aspects as well as statistics for case IV are under detailed investigation and will be reported later.

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## UPTAKE AND METABOLISM OF $^{14}\text{C}$ -LABELLED GLUCOSE IN *SARGASSUM ILICIFOLIUM*

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### ABSTRACT

*Sargassum ilicifolium*, a common marine brown alga, shows the ability to take up glucose and metabolise it. Metabolism is greater in light than in darkness. The main products of metabolism are sugar phosphates, glutamate, aspartate and alanine and to some extent citrate, fucose, mannose, fucoidin, laminarin and alginate. Alginic acid biosynthesis starts later and is not possibly from mannitol. The alga probably belongs to a group of  $\text{C}_4$  plants of aspartate formers.

MARINE algae are well known for synthesising a wide range of complex organic compounds. In order to study metabolic pathways of these organic compounds, several attempts<sup>1-3</sup> have been made to feed marine algae with labelled compounds which are suspected to be the precursors. In the

light of these observations, it was thought that an experiment of feeding uniformly labelled glucose- $^{14}\text{C}$  to *Sargassum ilicifolium* could be designed.

### MATERIALS AND METHODS

*S. ilicifolium* was collected at low tides from sea-shores of Ratnagiri and placed in sea water.

TABLE I

Distribution of total radioactivity in various compounds formed after supplying uniformly labelled  $^{14}\text{C}$ -glucose

(The activity represents c.p.m. in 4 g of fresh tissue. Activity in glucose spot is not considered even though counted)

| Name of compound              | 30 Minutes |      | 2 Hours |      | 6 Hours |      |
|-------------------------------|------------|------|---------|------|---------|------|
|                               | Light      | Dark | Light   | Dark | Light   | Dark |
| Sugar phosphates ..           | 2950       | 2100 | 4700    | 3550 | 5350    | 4150 |
| Aspartate ..                  | 2500       | 1800 | 4000    | 3700 | 4700    | 3900 |
| Glutamate ..                  | 7350       | 6350 | 5900    | 5150 | 6400    | 5150 |
| Glutamine ..                  | 1843       | 1254 | 2630    | 2718 | 3058    | 3128 |
| Alanine ..                    | 550        | 400  | 3300    | 3400 | 3250    | 4100 |
| Fucose ..                     | 650        | 325  | 872     | 764  | 976     | 816  |
| Mannose ..                    | 450        | 350  | 750     | 700  | 825     | 800  |
| Citrate ..                    | 1700       | 1850 | 2900    | 2400 | 3650    | 3100 |
| Fuccidin - Laminarian* ..     | 250        | 325  | 450     | 462  | 750     | 673  |
| Alginic acid* ..              | —          | —    | —       | —    | 460     | 390  |
| Other compounds* ..           | —          | —    | —       | —    | 265     | 262  |
| Other insoluble fractions* .. | —          | —    | —       | —    | 248     | 194  |

\* Ethanol insoluble fractions.

— Activity not recorded.

The plants were washed first in filtered sea water and then in synthetic sea water<sup>4</sup>. The experiments of 30 minutes, 2 hours and 6 hours were carried out in two sets either in light or in darkness. The samples weighing 4 g were cut into small squares. The algal material was taken in an Erlenmeyer flask and floated in 60 ml of 0.025 M glucose solution (pH 8.0) prepared in synthetic sea water. The reaction was initiated by adding 0.02 mc of glucose- $^{14}\text{C}$  (specific activity 280 mc/mM). Experiments under light were performed by supplying illumination of about 4000 ftc near the algal material. Temperature was maintained between 15° C and 20° C. The flask was continuously shaken. The reaction was terminated by the addition of boiling 80% ethanol.

The material was homogenised and the extract filtered. The residual solids were analysed according to the method described by Bidwell<sup>5</sup>. The radioactivity of individual insoluble fractions was determined.

The combined filtrate was evaporated under reduced pressure to 5 ml and electrolytically desalted. The extract was analysed for ethanol soluble fractions by two-dimensional paper chromatography employing phenol : water (80 : 20 v.v) and butanol : acetic acid : water (80 : 22 : 50 v.v.v) as solvents. The radioactive compounds were detected by exposing the chromatograms to X-ray films. The activity incorporated in individual compounds was counted with a transistorised proportional counting system and confirmed with a liquid scintillation system.

## RESULTS AND DISCUSSION

Table I represents soluble as well as insoluble fractions and their radioactivity as counts per minute in 4 g of fresh tissue. A close scrutiny of autoradiograms revealed that glucose was gradually utilized. The utilization of glucose was more in light than in darkness. As a result of the utilization of glucose, sugar phosphates, amino acids and organic acids could be detected in the soluble fraction. In the soluble fraction, besides glucose, two spots in the sugar area had also shown incorporation of radioactivity. In the soluble fraction, incorporation was slow and alginic acid had little incorporation and that too only after 6 hours, while fuccidin and laminarian incorporated  $^{14}\text{C}$  from 30 minutes onwards. The results can be favourably compared with those of Lin and Hassid<sup>3</sup>.

The results are on similar lines and indicate that in *S. ilicifolium* sugar phosphates are formed by glucose utilization. This will obviously require phosphorylation and it appears enzymes similar to those present in *F. gardneri* may also be operating in *S. ilicifolium*. No label could be detected in mannitol, which possibly indicates that glucose is not the precursor of this important sugar alcohol in the brown alga. This observation is similar to that of Lin and Hassid<sup>3</sup>.

The incorporation of radioactivity into glutamate, aspartate and alanine clearly indicates that these were the major amino acids formed. This is similar to the result obtained by Bidwell and Ghosh<sup>2</sup>. From the recent unpublished work on photosynthesis of *Sargassum*, a good amount of radioactivity

was located in these amino compounds. Bidwell and coworkers<sup>4</sup> found out that in wheat leaves, when radioactive glucose was supplied, alanine became much more radioactive in light than in darkness. This was attributed to blockage of the carbohydrate respiration. It was possible for them to reverse blockage by the application of amino acids intrusion. The observation of more radioactivity in darkness is similar to that of Bidwell *et al.*<sup>5</sup>. However, whether it is respiration blockage cannot be ascertained with the present data.

Bidwell and Ghosh<sup>2</sup> have found that uniformly labelled glucose <sup>14</sup>C is utilized by *F. vesiculosus*. They have indicated several pathways of glucose utilization involving carboxylation and decarboxylation reaction. They notice that organic acids are better substrates for alginic acid than glucose. These authors also did not record any activity in mannitol but recorded it in fucose. From Table I, it is clear that activity in fucose fraction is higher at 6 hours than at half an hour. It is of interest to record the highest activity in citrate as noted by Bidwell and Ghosh<sup>2</sup>. However no explanation for this can be given with the present data. From Table I, it is also clear that alginic acid synthesis starts much later. This observation indicates that in *Sargassum* alginic acid biosynthesis starts later and

it is possibly from sugar phosphates as proposed by Lin and Hassid<sup>3</sup>.

In the present investigation, it is of interest to record an appreciably high label in aspartate and no label in malate. This, in the light of recent work of Karekar and Joshi<sup>7</sup>, indicates that *S. ilicifolium* belongs to a group of C<sub>4</sub> plants of aspartate formers and possibly aspartate is a key compound from which many other metabolic products are formed.

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#### PRELIMINARY OBSERVATIONS ON THE USE OF MARINE CATFISH PITUITARY GLANDS FOR INDUCED SPAWNING OF THE INDIAN MAJOR CARPS

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#### ABSTRACT

Major carps are not available in adequate numbers, especially along the coastal regions of the country, for collection of pituitary glands for large scale induced spawning of these species. Therefore, attempts were made to utilise the pituitary of marine catfish, which are landed in good numbers along the coastal areas, for induced spawning of the major carps. Two species of the major carps, namely, *Labeo rohita* and *Cirrhina mrigala*, were successfully spawned during July, 1973 by administering intramuscular injections of pituitary extract of two species of marine catfish, *Tachysurus thalassinus* and *T. jella*. Sixtyfour per cent of the treated carps spawned successfully when injected with 30 mg and 20 mg of marine catfish pituitary per kg body weight of female and male fish respectively.

#### INTRODUCTION

THE Indian major carps were successfully induced to breed by the administration of fish pituitary hormones in 1957 (Chaudhuri and Alikunhi, 1957; Alikunhi *et al.*, 1960; Chaudhuri, 1960). Since then, the induced breeding technique has been vastly improved and its use as a dependable method of pure fish seed production has spread

widely to all States of India. However, the quantity of fish seed produced in the country through this method in 1964-65 was only 1.57% of the total annual production (Anon., 1966). One of the reasons for this low percentage of fish seed produced through hypophysation technique is the shortage of carp pituitary glands, especially along the coastal areas. With a view to overcome this limitation, studies on the use of marine catfish pituitary glands for induced breeding of freshwater fishes were initiated at the College of Fisheries, Mangalore, in

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1972, when a freshwater catfish, *Clarias batrachus*, was spawned successfully by injection of pituitary extracts from a marine catfish, *Tachysurus* sp. (Devaraj *et al.*, 1972). Experiments were conducted to induce breeding in the major carps, *Cirrhina mrigala* and *Labeo rohita*, with injections of pituitary extracts from two species of marine catfish during July, 1973 and the results thereof are reported in this communication.

#### MATERIALS AND METHODS

The pituitary glands used for the experiments were collected from the marine catfish, *Tachysurus thalassinus* and *T. jella* caught off the South Kanara Coast. The glands were collected during the period 30-1-1973 to 2-2-1973, from specimens ranging in weight from 1.0 kg to 9.75 kg. About 97% of the donor catfish were in the I or II stages of sexual maturity. The glands were preserved in absolute alcohol and stored in a refrigerator.

The induced breeding experiments were conducted in the fish farm of the Tungabhadra Board (Karnataka State) during July 1973. The major carp brood fish used for the experiments were all farm-reared and belonged to the age groups of II to IV. The methods followed for the preparation of pituitary extract and administration of hormone were the same as usually practised for the breeding of major carps.

The water temperature during the experimental period ranged from 25.3°C to 27.7°C. The weather was mostly cloudy, with one or two showers during most of the days.

#### RESULTS

The results of the induced spawning experiments are presented in Table I. Seven females and eleven males of *mrigal* were treated with pituitary extract of the marine catfish. In all cases, except in one set, the female and male fish received a total of 30 mg/kg and 20 mg/kg body weight of pituitary glands respectively. In experiment No. 4, one set of *mrigal* was injected at a lower dose of 20 mg/kg and 15 mg/kg pituitary for the female and male fish respectively. Of the six *mrigal* which received the higher dose of pituitary, five spawned successfully, yielding over 20 lakhs of eggs with normal rates of fertilisation. The *mrigal* treated at a dose of 20 mg/kg did not spawn. During these experiments, it was seen, that pituitary extract prepared and kept in a refrigerator for 24 hours could also be successfully used. Along with the fish injected with the marine catfish pituitary, six females and eleven males of *mrigal* were also treated with carp pituitary extract in order to compare the results. Of these, four spawned successfully yielding a total of 16,28,000 eggs.

From Table I it is seen that all *rohu* females and males, except one set in experiment No. 7, received 30 mg/kg and 20 mg/kg body weight of catfish pituitary respectively. One set of *rohu*, in experiment No. 7, was injected at a lower dose of 20 mg/kg and 15 mg/kg body weight of pituitary for female and male fish respectively. Of the five *rohu* treated at the higher dose, two spawned successfully yielding 7,54,600 eggs, while that injected with the lower dose did not spawn. Along with this, four *rohu* administered carp pituitary hormones also spawned.

#### DISCUSSION

The present experiments clearly demonstrate that it is possible to use the pituitary of the marine catfish *T. thalassinus* and *T. jella*, for induced spawning of the Indian major carps. From Table I, it is clear that seven out of eleven females treated with the marine catfish pituitary extract at a dose of 30 mg/kg spawned. Two female fish, one *rohu* and one *mrigal*, injected at a lower dose of 20 mg/kg of pituitary, did not spawn. Among the ten control fish treated with carp pituitary, eight spawned, accounting for 80% of positive results.

Normally doses of 6 to 10 mg/kg for female and 4 to 6 mg/kg body weight for male fish are used for induced spawning of the Indian major carps, when pituitary extracts from mature carps are injected. The dosage of pituitary required to spawn major carps when the donor carps are immature is not known. During the present experiments it was not possible to work out the minimum threshold dose of pituitary of the marine catfish required for spawning of major carps. A higher dose of pituitary of the marine catfish was used deliberately in view of the immature condition of the donor fish and the phylogenetic and habitat differences between the donor and recipient species. Though it is reported that there is no great phylogenetic specificity between the pituitary hormones of different species of fishes (Pickford and Atz, 1957), some of the heteroplastic injections have failed to yield positive results. For instance, Clemens and Sneed (1962) have reported that extremely high doses of carpsucker (*Carpiodes carpio*) pituitaries were required to ovulate female goldfish. The Indian major carps could not be spawned by pituitaries of grey mullets (*Mugil cephalus* and *Liza troschelli*) and *Tilapia mossambica* (Anon., 1970). In view of the fact that the donor catfish in our experiments were mostly immature, it should be possible to spawn the major carps with a much lower dose by employing pituitaries of mature marine catfish.

On an average, the catfish, mainly represented by various species of *Tachysurus*, accounts for

TABLE I

Dosage and response of brood fish injected with the marine catfish or carp pituitary glands

| Expt. No. | Species | Injected with marine catfish pituitary   |                      |   | Injected with carp pituitary (controls)  |                |  |
|-----------|---------|--|----------------------|---|--|----------------|--|
|           |         | Wt. of brood fish (kg)   | Dosage (mg/kg)       | Response (Remarks)  | Wt. of brood fish (kg)   | Dosage (mg/kg) | Response (Remarks)   |
| 1.        | Mrigal  | F 2.00<br>M 1.00 }<br>M 0.70 }   | 30<br>20             | +   | F 2.00<br>M 0.50 }<br>M 0.50 }<br>M 0.50 }   | 4.5<br>4.0     | +  |
| 2.        | Mrigal  | F 1.50 }<br>F 1.00 }<br>F 0.50 }<br><br>M 0.75 }<br>M 0.50 }<br>M 0.50 }<br>M 0.50 } | 30<br>20             | +<br>+ Smaller ♀ got<br>- 'plugged'   | F 3.00 }<br>F 1.50 }<br>F 2.00 }<br><br>M 2.50 }<br>M 1.00 }<br>M 0.50 }<br>M 2.00 } | 6.0<br>4.0     | +<br>- The ♀ which<br>+ did not spawn<br>[ got 'plugged' ] |
| 3.        | Mrigal  | F 3.00<br>M 2.50 }<br>M 2.00 }   | 30<br>20             | +   | F 3.00<br>M 2.50 }<br>M 2.00 }   | 7.5<br>5.0     | -  |
| 4.        | Mrigal  | F 4.00<br>F 4.00<br>M 2.00<br>M 2.00 }<br>M 1.00 }                                   | 30<br>20<br>15<br>20 | +<br>-  | F 4.50<br>M 1.00 }<br>M 1.00 }   | 5.5<br>4.0     | +  |
| 5.        | Rohu    | F 1.00 }<br>F 0.50 }<br>F 0.50 }<br>M 0.50 }<br>M 0.50 }<br>M 0.50 }<br>M 0.50 }     | 30<br>20             | +<br>- One ♀ got<br>- 'plugged'.<br>Third one jumped<br>out of the<br>breeding hapa | F 2.00<br>M 1.00 }<br>M 1.00 }<br>M 0.50 }   | 7.5<br>5.0     | +  |
| 6.        | Rohu    | F 4.00<br>M 2.00 }<br>M 1.00 }   | 30<br>20             | +   | F 4.00<br>M 2.00 }<br>M 1.50 }   | 7.5<br>5.0     | +  |
| 7.        | Rohu    | F 2.50<br>F 4.00<br>M 3.00<br>M 2.50 }<br>M 2.00 }                                   | 30<br>20<br>15<br>20 | -<br>-  | F 5.00 }<br>F 4.00 }<br>M 2.50 }<br>M 2.50 }<br>M 2.50 }                             | 7.5<br>5.0     | +<br>+   |

F = Female; + = Positive response.

M = Male; - = Negative response.

about 5% of the total marine fish landings of India. As the bulk of the catfish landed are used for sun-drying, for which the fish are split open, the fishermen may not object if the scalp is cut for the collection of pituitary glands. The cost of a major carp pituitary gland works out to about 50 paise, while it is only 10 paise in the case of marine catfish gland. Moreover, the major carps are scarce along most of the coastal areas. In this context,

the present finding should help not only in reducing the cost of fish breeding work, but also to solve the problem of shortage of carp pituitary at fish seed production centres.

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## RECENT TRENDS IN ELECTROCHEMICAL SCIENCE AND TECHNOLOGY

THE Society for Advancement of Electrochemical Science and Technology (SAEST) is a professional scientific body which was founded in 1965 at Karaikudi. It was started with a view to disseminating knowledge on Electrochemical Science and Technology. It publishes two journals, one quarterly Journal titled *Transactions of SAEST* and the other monthly *Current Titles in Electrochemistry*. In addition, the SAEST arranges joint symposia in collaboration with Electrochemical Industries, and these are held at the premises of the sponsoring industries. It also organizes Technical sessions every year on topics of current interest in the field of electrochemical science. To mark the completion of 10 years of its useful service, the Society observed its Decenary Celebrations on 26–27th November 1974 at Karaikudi.

A technical session on 'Recent Trends in Electrochemical Science and Technology' was got up for this occasion and it was primarily devoted to survey the status of Electrochemical Industries and their future prospects with particular reference to India. The recent developments in the field of Electrochemical Technology in India were also included in the scope of the session. The subjects covered were Chlor-alkali, Batteries, Electro-metallurgy, Electro-thermal products, electro-organic and inorganic chemicals, Metal finishing, and Anti-corrosion products. The venue for this seminar was Central Electrochemical Research Institute, Karaikudi, where the headquarters of the SAEST is located.

The symposium was organized under five sessions. In the first session on "Electro-organic and inorganic products", articles on 'Chloralkali Industry in India,

'Titanium Substrate Insoluble Anode for Chlor-alkali Cells', 'Inorganic Electrochemicals in India' and 'Electro-organic Products' were presented. The second session was devoted to 'Electrometallurgy' papers covering Lead, Zinc, Copper and Nickel. The third session on 'Electrothermics and fused salts' presented discussions on a number of electro-thermal products. During the fourth session, an account of the technology, production and growth of the Indian battery industry was presented. During the last session, the present Status and Modern Trends in Phosphating, a prepainting stage in the treatment for metals were discussed.

Three special lectures also formed part of the technical session. Prof. S. Ramaseshan gave a talk on 'Electrochemical Machining' describing the work currently carried out at the National Aeronautical Laboratory, Bangalore.

Prof. S. K. Rangarajan of National Aeronautical Laboratory, Bangalore, in his lecture on 'Perspectives in Fundamental Investigations' presented the basic approaches for evaluating typical problems of technological importance such as dendritic growth of zinc, optimization of cell design in electrochemical reactors. Finally Prof. K. S. G. Doss, the Founder President and former Director of Central Electrochemical Research Institute lectured on the principles of metal corrosion.

The session was attended by well over 230 delegates from all over the country including a number of representatives from industries and Governmental bodies and brought to focus the recent developments, existing problems and future projections relating to electrochemical industries in India.

## INTERNATIONAL SYMPOSIUM ON BIOLOGY AND MANAGEMENT OF MANGROVES

**A**N International Symposium on the Biology and Management of Mangroves was held at East-West Centre, Honolulu, Hawaii (U.S.A.) from October 8 to 11, 1974. It attracted participants and observers from all over the world including UNESCO. There were 13 sessions which dealt with general aspects of mangroves, geomorphology, biogeography, soils, chemical elements, micro-organisms, physiology, estuaries, communities, systems and synthesis. The management aspects were also covered. About 70 papers were read and discussed.

An interesting feature of the Symposium was the formation of small working groups from among participants. The groups were assigned to list priorities in mangrove research and management. The recommendations of the various groups were gathered, processed and forwarded to the UNESCO-SCOR for further action. A proposal was mooted for an international society for the study of mangroves, and later, promotion of a journal to bring various mangrove-related publications under one roof. It is hoped that this proposal will be taken up seriously in due course.

The Symposium concluded with passing a resolution addressed to the Secretary-General, U.N.O.

It emphasized the catholicity of world's mangrove ecosystems as "valuable resources and .... governments be urged to promote research to better appraise these ecosystems and .... exert special care when evaluating actions or proposals that could commit these resources".

We are indebted to Dr. A. G. Pawar, Vice-Chancellor, Shivaji University, Dr. S. P. Adinarayana, Vice-Chancellor, Annamalai University and Dr. R. Natarajan, Director, Centre of Advanced Study in Marine Biology of Annamalai University, Porto Novo, for their keen support and encouragement; and to the authorities of the respective Universities for permission to their staff to participate as delegates in the Symposium.

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Marine Biological Station of  
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Porto Novo 608 502, Tamil Nadu,  
November 23, 1974.

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S. R. S. SASTRY  
Manager  
Current Science Association



## LETTERS TO THE EDITOR

ANALYTICAL APPLICATIONS OF 5-ETHYL  
RESACETOPHENONE OXIME (5-ERAPO)

## Gravimetric Determination of Copper

5-ETHYL resacetophenone oxime (5-ERAPO) has been found to be a suitable chelating agent for gravimetric determination of copper. The reagent reacts quantitatively with Cu(II) in the pH range 3.5 to 9.0 giving an insoluble metal complex. The buff coloured copper complex can directly be weighed as  $\text{Cu}(\text{C}_{10}\text{H}_{12}\text{O}_3\text{N})_2$  after drying at  $110^\circ$  to  $120^\circ\text{C}$ , the error being less than  $\pm 0.3\%$ . The reagent gives pale green precipitate with Ni(II) at pH 5.0, orange yellow precipitate with Co(II) at pH 6.5 and violet colour with Fe(III) at pH 2.5 to 4.5. The interference due to several ions such as Ca(II), Ba(II), Sr(II), Mg(II), Ni(II), Co(II), Zn(II), Al(III) has been avoided by working at controlled pH, and by using suitable masking agent. The accuracy in the above case is  $\pm 0.7\%$ .

The reagent<sup>1</sup> was prepared as follows:

4-Ethyl resorcinol (7.5 gm), obtained by the Clemmensen's reduction of resacetophenone, was heated with fused zinc chloride (20 gm) in glacial acetic acid (30 ml). The reaction mixture was boiled and then cooled to room temperature. This reaction mixture was cooled with ice after the addition of 100 ml of cold 50% HCl. The product 5-ethyl resacetophenone (5.2 gm) was crystallised from hot benzene (M.P.  $119^\circ\text{C}$ ), its oxime was prepared by the usual method and crystallised from hot water (M.P.  $141^\circ\text{C}$ ).

The copper bischelatate  $\text{Cu}(\text{C}_{10}\text{H}_{12}\text{O}_3\text{N})_2$  (Cu calculated 14.06%, found 13.92%) with the reagent precipitated at pH 3.5 is buff coloured and is insoluble in water, 40% ethanol, ether and dilute acetic acid; but it is soluble in chloroform and ethanol.

**Procedure for determination of Cu(II).**—An aliquot standard solution of Cu(II) containing 15 to 63 mg metal was diluted to 200 ml and pH was adjusted using acetate buffer. The solution was warmed to  $60^\circ$  and 1.0% ethanolic solution of the reagent was added with constant stirring till it was 10% excess over the calculated amount. The precipitate was digested at about  $60^\circ$  and allowed to stand for about 30 min. It was filtered through a sintered glass crucible (G4), washed with 40% ethanol to remove excess reagent, dried at  $110^\circ$  to  $120^\circ\text{C}$  to constant weight and finally weighed as  $\text{Cu}(\text{C}_{10}\text{H}_{12}\text{O}_3\text{N})_2$ . The gravimetric factor

(copper/copper complex) is 0.1407. The results are given in Table I.

TABLE I  
Determination of copper

| Cu(II) mg<br>(taken) | Cu(II) mg<br>(found) | %<br>(error) |
|----------------------|----------------------|--------------|
| 7.85                 | 7.80                 | -0.64        |
| 15.71                | 15.68                | -0.19        |
| 31.43                | 31.50                | +0.22        |
| 47.14                | 47.24                | +0.21        |
| 62.86                | 62.72                | -0.22        |

**Determination of copper in presence of other ions.**—The pH range at which the precipitation of Ni(II) and Co(II) takes place is higher than that for the precipitation of Cu(II). It was therefore possible to determine Cu(II), (30 mg) at pH 3.5 in the presence of an equal amount of Ni (% error 0.2) and Co (% error 0.7). Zn(II), Mg(II), Ba(II), Ca(II), Sr(II) were found to give no colour nor an insoluble chelate. Hence copper could be determined in presence of these ions with a maximum error of 0.7%. Ferric (III) interferes during the precipitation of copper (II); however the determination was carried out sequestering Fe(III) by potassium citrate.

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REACTION ON BIS-FORMAMIDINE  
DISULPHIDE WITH INDOLE

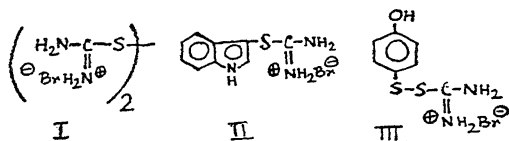
WITH a view to extending the reaction<sup>1</sup> of bis-formamidine disulphide dihydrobromide I, to reactive aromatic systems other than phenols, the reaction of I with indole was investigated. Indole 1.2 g was heated with 3.1 g of I at  $60^\circ$  for 30 minutes. The reaction mixture was cooled, triturated with water and filtered when a dry white hydrobromide 1.9 g (70%) was obtained, crystals from warm, dil. HBr, m.p.  $217-219^\circ$  (decomp.) (Found: C, 40.12; H, 4.08; N, 15.11%. Required for  $\text{C}_9\text{H}_{10}\text{N}_3\text{SBr}$ : C, 39.70; H, 3.67; N, 15.44%).

The n.m.r. spectrum of the hydrobromide in  $D_2O$  n.m.r. frequencies showed multiplets as expected, in 7.3–7.9  $\delta$  region for all the aromatic protons of indole, the other protons attached to nitrogen showing no peaks due to exchange with  $D_2O$ .

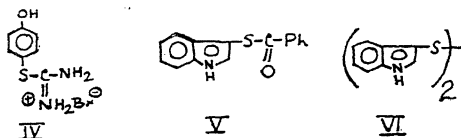
The most probable site in indole for attack of the sulphenium ion,  $\oplus S-C-NH_2$ , is the 3 position,



resulting in the formation of the hydrobromide II. That the hydrobromide was not a disulphide of the type III could be seen by its lack of reactivity aq. KI and aq.  $H_2S$  solution<sup>1</sup>.



The II on treatment with cold dil. alkali immediately precipitated a pale yellow solid which darkened on exposure to air. The sulphides of the type IV are known<sup>1</sup> to decompose in alkaline solution to thiols, which slowly get oxidised to disulphides. The dark coloured solid from II had m.p. 90–110°; the thiol from the corresponding hydriodide of II obtained under nitrogen atmosphere is reported<sup>2</sup> to melt at 99–100°. The dark solid (the m.p. of which kept rising on keeping) on shaking with  $H_2O_2$  for an hour, gave a white solid; crystallised from dil. ethanol, m.p. 214–217°; reported<sup>2</sup> m.p. for disulphide VI is 218–220°.



The II on Schotten Baumann benzoylation gave a pale yellow crystalline benzoate V, m.p. 144–145° (from ethanol), (Found S, 12.03; calc. for  $C_{15}H_{11}NOS$  12.64%); the reported<sup>2</sup> m.p. for the benzoate V is 142–144°. That the sulphur was linked at the 3 position of the indole was demonstrated by the earlier workers<sup>3</sup>.

Thanks of the author are due to the authorities of Banaras Hindu University for providing the facilities and to the C.S.I.R. for the award of Post Officership (1971–73).

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### ANALYTICAL APPLICATIONS OF 2-AMINO-5-NITROSO-4, 6-PYRIMIDINEDIOL

CONTINUING the work<sup>1,2</sup> on analytical applications of pyrimidinols, in the present note the potentialities of the compound 2-amino-5-nitroso-4, 6-pyrimidinediol (ANP) are described. ANP reacts with  $Fe^{2+}$  and  $Ru^{3+}$  giving respectively blue and pink colorations suitable for their determination. The yellow complex obtained with  $Co^{2+}$  solution is also of analytical interest.

ANP was obtained from K & K Laboratories, U.S.A. Its standard solution (0.004 M) was prepared by dissolving 624 mg of the compound in 1 litre of double distilled water (hot). The stock solutions of  $Fe^{2+}$ ,  $Ru^{3+}$  and  $Co^{2+}$  were prepared by dissolving appropriate amounts of Mohr's salt in double distilled water, ruthenium chloride in 1 M-HCl and cobalt metal in dilute HCl. To Mohr's salt solution, few ml of 1% hydroxylamine hydrochloride were added to prevent oxidation of  $Fe^{2+}$  to  $Fe^{3+}$ . The solutions were standardized by gravimetric methods.

The aqueous solution of ANP is light red in colour, absorbing maximum at 255 ( $\epsilon = 2800$ ) and 315 nm ( $\epsilon = 840$ ). The characteristics of the complexes formed with  $Fe^{2+}$ ,  $Ru^{3+}$  and  $Co^{2+}$  salt solutions are summarized in Table I. Because of the intensity of the colours of iron (III) and ruthenium complexes, they were used in spectrophotometric determination of the two metals.

**Procedure for determination of iron.**—To a suitable aliquot of iron (II) solution, add 5.0 ml of ANP solution followed by 4.0 ml of sodium acetate-hydrochloric acid buffer solution (pH 5.0). Make up the volume and measure the absorbance of the blue solution at 640 nm against reagent blank. Calculate the amount of iron by comparing the absorbance with a calibrated curve.

**Procedure for determination of ruthenium.**—To a suitable aliquot of ruthenium solution, add 8.0 ml of ANP solution followed by 4.0 ml of acetate buffer (pH 5.0). Heat the solution on steam bath for one hour, cool it and make up the volume. Note the absorbance of the pink solution at 517 nm

TABLE I  
Characteristics of metal complexes with ANP

| Characteristic                                  | Fe <sup>2+</sup> -complex | Ru <sup>2+</sup> -complex | Co <sup>2+</sup> -complex |
|---|---------------------------|---------------------------|---------------------------|
| Colour  | .. Blue                   | Pink                      | Yellow                    |
| $\lambda_{\text{max}}$ (nm)                     | .. 640                    | 517                       | 360                       |
| $\epsilon_{\text{max}}$                         | .. 19,000                 | 17,000                    | 78,500                    |
| ANP needed for full colour development          | .. 50 times               | 90 times                  | 20 times                  |
| pH range for maximum absorbance                 | .. 4.5-7.3                | 3.9-5.7                   | 3.5-6.4                   |
| Beer's law range ( $\mu\text{g/ml}$ )           | .. Up to 2.7              | Up to 3.5                 | Up to 1.4                 |
| Sandell's sensitivity <sup>3</sup>              | .. 0.0033                 | 0.0059                    | 0.0008                    |
| Composition (metal : ANP)                       | .. 1:3                    | 1:3                       | 1:3                       |
| Apparent instability constant                   | .. $6.9 \times 10^{-14}$  | $6.4 \times 10^{-14}$     | $5.8 \times 10^{-17}$     |
| Standard deviation (with 8 samples)             | .. 0.0043                 | 0.0047                    | 0.0037                    |
| Relative mean deviation (in parts per thousand) | .. 9.6                    | 12                        | 4.8                       |
| Coefficient of variation                        | .. 1.16                   | 1.40                      | 0.59                      |

against the reagent blank prepared in the same way. Calculate the amount of ruthenium in the sample by comparing its absorbance with a standard curve.

In determination of 1.1  $\mu\text{g/ml}$  of iron, the following ions could be tolerated to the limits given in parentheses (in  $\mu\text{g/ml}$ ):  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CNS}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , phthalate or thiourea (1,000);  $\text{S}_2\text{O}_3^{2-}$  (500);  $\text{NO}_2^-$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{UO}_2^{2+}$  or  $\text{MoO}_4^{2-}$  (100);  $\text{Mn}^{2+}$  (75);  $\text{F}^-$ ,  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Pb}^{2+}$  or  $\text{Cr}^{3+}$  (50);  $\text{C}_4\text{H}_4\text{O}_6^{2-}$  (20);  $\text{PO}_4^{3-}$  or  $\text{Ni}^{2+}$  (10);  $\text{As}^{3-}$  (5). Serious interference was observed from  $\text{C}_2\text{O}_4^{2-}$ , EDTA, citrate,  $\text{BO}_3^{3-}$ ,  $\text{Cu}^{2+}$ ,  $\text{V}^{3+}$ ,  $\text{V}^{5+}$ ,  $\text{Fe}^{3+}$  or  $\text{Co}^{2+}$ .

In determination of 1.0  $\mu\text{g/ml}$  of ruthenium, the following ions could be tolerated to the extents given in parentheses (in  $\mu\text{g/ml}$ ):  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$  or  $\text{CH}_3\text{COO}^-$  (1,000);  $\text{NO}_3^-$  or  $\text{ClO}_4^-$  (50);  $\text{C}_2\text{O}_4^{2-}$  (100),  $\text{Zn}^{2+}$  (75),  $\text{Al}^{3+}$  (50),  $\text{C}_4\text{H}_4\text{O}_6^{2-}$  or  $\text{PO}_4^{3-}$  (40);  $\text{SO}_4^{2-}$  (15); citrate or  $\text{Pb}^{2+}$  (10);  $\text{F}^-$ , EDTA or  $\text{Pd}^{2+}$  (2-4). Serious interference was observed from  $\text{CNS}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_3^{2-}$ ,  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{BO}_3^{3-}$ , thiourea,  $\text{Ag}^-$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Sn}^{2+}$ ,  $\text{As}^{3-}$ ,  $\text{Sb}^{3-}$ ,  $\text{Bi}^{3-}$ ,  $\text{V}^{4+}$ ,  $\text{V}^{5+}$ ,  $\text{Cr}^{3+}$ ,  $\text{MoO}_4^{2-}$ ,  $\text{UO}_2^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Rh}^{3+}$ ,  $\text{Os}^{5+}$  or  $\text{Ir}^{3+}$ .

The present work is done under the U.G.C. scheme "Environmental Chemistry".

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#### INTERACTION OF BILIRUBIN WITH HUMAN ERYTHROCYTE MEMBRANE\*

BILIRUBIN reaches toxic levels in icteric newborns (hyperbilirubinemic newborns) especially in "Kernicterus" and causes irreversible nuclear damage of brain and other tissues<sup>1,2</sup>. It has been shown earlier that bilirubin interferes with the fragility of human erythrocytes<sup>3</sup>. The present report deals with the binding of bilirubin to human erythrocyte membranes.

Tritium labelled bilirubin prepared by Wilzbach procedure as described by Grodsky *et al.*<sup>4</sup> was supplied by Isotope Division of Bhabha Atomic Research Centre, Trombay, India. The preparation was purified to constant specific activity (7  $\mu\text{Ci}/\mu\text{mol}$ ) as described by Kapoor *et al.*<sup>5</sup>. Washed erythrocytes were prepared from the blood of healthy donors from the Blood Bank of K.G.'s Medical College, Lucknow<sup>6</sup>. Human erythrocytes ( $1 \times 10^9$  cells) were incubated in a metabolic shaker 40 strokes/min (ampl: 2 cm) at 25°C for 30 min, in a 3 ml incubation medium containing Tris HCl buffer pH 7.5 (8.1 mM) and sodium phosphate buffer pH 7.5 (8.1 mM) with varying concentrations of <sup>3</sup>H-bilirubin, in presence or absence of different amounts of albumin precalculated to give varying bilirubin : albumin molar ratios.

After incubation cells were washed free from media and dispersed in hypotonic sodium phosphate buffer pH 7.5, incubated at 5°C for 30 min and

centrifuged at  $20,000 \times g$  at  $5^\circ C$  for 30 min. The ghosts containing bilirubin were dispersed in hypotonic buffer and the suspension again centrifuged at  $20,000 \times g$  for 30 min. The supernatant fluid was recovered carefully by aspiration.  $^3H$ -bilirubin was extracted from erythrocyte ghosts and hemolysate with chloroform and methanol (2:1 v/v) and dried *in vacuo*. Aliquots of dried samples were suspended in vial containing 15 ml of scintillation fluid 0.4% (w/v) 2,5, diphenyl oxazol (POP) and 0.04% (w/v) [1-4-bis-2-(Methyl-5-phenyl-oxazolyl)-benzene] (POPOP) in distilled toluene, and the radioactivity of the samples measured in low  $K^+$  vials. The efficiency of the Packard Tricarb Scintillation Spectrometer was of the order of 40 to 43% for  $^3H$  as measured by using *n*-hexadecane 1.2 T (Amersham, Bucks, U.K.) as internal standard.

In blood, bilirubin is firmly bound to serum proteins relatively strongly to albumin<sup>6</sup>. This mechanism protects the cells as long as the molar ratio of bilirubin to albumin is less than 1:17.8. Since the determination of erythrocyte bound bilirubin could be of clinical significance in cases of neonatal jaundice<sup>9-10</sup>, attempts were made to confirm the interaction of bilirubin with erythrocytes by conducting binding studies with  $^3H$ -bilirubin and erythrocyte at different molar ratios of bilirubin to albumin. The results presented in Table I

TABLE I

*Binding of bilirubin to erythrocytes at different molar ratios of bilirubin to albumin*

Erythrocyte ( $1.0 \times 10^9$  cells) were incubated in 3 ml incubation medium (pH 7.5) with different molar ratios of bilirubin and albumin at  $25^\circ C$  for 30 min in a metabolic shaker. The total  $^3H$ -bilirubin concentration was kept constant ( $0.2 \mu$  mole containing  $55.5 \times 10^3$  cpm). After incubation cells were washed free from medium and bilirubin bound to erythrocytes were extracted and radioactivity of samples were counted in Packard Tri-Carb Scintillation Counter as described in the Method.

| Molar bilirubin/<br>albumin ratios | cpm $\times 10^{-3}/10^9$ cells |                         |
|------------------------------------|---------------------------------|-------------------------|
|                                    | Human serum<br>albumin          | Bovine serum<br>albumin |
| 0.5                                | 9.33                            | 12.27                   |
| 1.0                                | 10.12                           | 14.29                   |
| 1.5                                | 13.24                           | 19.30                   |
| 2.0                                | 18.17                           | 22.03                   |

indicate that greater binding of bilirubin to erythrocytes takes place, when molar ratio of bilirubin to albumin exceeds 1:1. However, a small amount of bilirubin is associated with erythrocytes

even at a molar ratio below 1:1. Human serum albumin (HSA) is more effective in protecting the binding of  $^3H$ -bilirubin to erythrocytes than bovine serum albumin (BSA), i.e., due to greater binding capacity of HSA for bilirubin. The association of bilirubin at molar ratio below 1:1 of bilirubin to albumin can be due to a finite number of "attachment sites" on human erythrocyte surface for albumin<sup>11</sup>. From studies of the pattern of distribution of  $^3H$ -bilirubin, picked up by erythrocytes between organelles and cytosol, more than 85% bilirubin taken up by erythrocytes appears in membrane fraction (Table II). By exhaustive

TABLE II

*Distribution of  $^3H$ -bilirubin in membrane and soluble fraction of erythrocyte*

Human erythrocytes ( $1.0 \times 10^9$  cells) were incubated in increasing  $21$ – $170 \mu M$  ( $17.3 \times 10^3$ – $13.8 \times 10^4$  cpm) concentration of  $^3H$ -bilirubin in 3 ml incubation medium (pH 7.5) at  $25^\circ C$  for 30 min, washed free from medium and  $^3H$ -bilirubin in erythrocyte ghosts and hemolysate was extracted with a mixture of chloroform and methanol (v/v 2:1); the solvent extract was dried *in vacuo* and radioactivity of the dry samples counted in vials containing scintillation fluid in a Pack Card Tri-carb Scintillation Spectrometer as described in Experimental Section.

| Bilirubin<br>concentra-<br>tion ( $\mu M$ ) | cpm $\times 10^{-3}/10^9$ cells |   |                           |   |
|---|---------------------------------|---|---------------------------|---|
|   | Erythrocyte<br>membrane         |   | Erythrocyte<br>hemolysate |   |
|   | None                            | In presence<br>of $10 \mu$<br>mole<br>glucose | None                      | In presence<br>of $10 \mu$<br>mole<br>glucose |
| 21  | 8.9                             | 12.5  | 1.3                       | 2.2   |
| 42  | 13.6                            | 17.9  | 1.6                       | 2.6   |
| 85  | 52.2                            | 56.2  | 3.6                       | 2.6   |
| 127   | 71.5                            | 81.5  | 3.0                       | 2.5   |
| 170   | 91.2                            | 101.7   | 3.0                       | 3.5   |

extraction of erythrocyte ghosts with mixture of chloroform : methanol (2:1 v/v) almost all the radioactivity was recovered along with the lipid and the residual ghost had only negligible radioactivity. These results indicate that sites are located on erythrocyte membrane and these account for the binding of bilirubin to erythrocyte even at molar ratio below 1:1 of bilirubin to albumin. Evidently, lipids are responsible for the binding.

The author wishes to record his appreciation to Dr. C. R. Krishna Murti for most valuable guidance and many thoughtful suggestions. Thanks are due to Mr. S. Dey and Dr. S. K. Roy of Endocrinology

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\* Communication No. 1978 from Central Drug Research Institute, Lucknow 226001.

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### LYSOGENIC CONVERSION IN *SERRATIA MARCESCENS*

LYSOGENISED cells sometimes show modification of one or more host properties. The production of toxin by *Corynebacterium diphtheriae* is perhaps the most striking example. The presence of a particular prophage in *B. megaterium* has been reported to modify colonial morphology<sup>2</sup>. In the genus *Salmonella*, the capacity to form new somatic antigens is conferred by presence of certain prophages. Loss of the antigenic determinants is always found to be associated with loss of the prophage<sup>3</sup>. Phage conversion involving an alteration of somatic antigens has also been reported in *Pseudomonas aeruginosa*<sup>4</sup>. In *Staphylococci*, alterations in phage typing pattern, susceptibility to penicillin and the capacity to produce toxin are affected by lysogenization with appropriate phage<sup>5</sup>. In *Bacillus cereus* lysogeny and toxinogeny also appear to be interdependent<sup>6</sup>.

Lysogenic conversion resulting in altered enzymic activity has been reported in *Mycobacterium smegmatis*<sup>7</sup>.

In the present investigation we report changes in pigment production by nonlysogenic *Serratia marcescens* versus *Serratia marcescens* lysogenised by phage kappa.

TABLE I

Phage resistance pattern of lysogenic and non-lysogenic strains of *S. marcescens*

| Strain       | Plaque count per ml |
|--------------|---------------------|
| Nonlysogenic | $5.4 \times 10^8$   |
| Lysogenic    | Nil                 |
| Nonlysogenic | $1 \times 10^8$     |
| Lysogenic    | Nil                 |
| Nonlysogenic | $5.2 \times 10^8$   |
| Lysogenic    | Nil                 |

The original phage suspension was diluted in phosphate buffer and then 0.1 ml of the diluted suspension taken for plaque count on nutrient agar using as the plating organism 1 ml of 24 hrs culture of *S. marcescens* and its lysogenic strain.

TABLE II

Effect of U-V exposure on the lysogenic and non-lysogenic strains of *S. marcescens*

| Time of exposure | Plaque count per ml |              |
|------------------|---------------------|--------------|
|                  | Lysogenic           | Nonlysogenic |
| 30 sec           | $120 \times 10^4$   | Nil          |
| 1 min            | $140 \times 10^6$   | "            |
| 5 min            | $210 \times 10^6$   | "            |
| 15 min           | $790 \times 10^6$   | "            |
| 30 min           | $122 \times 10^7$   | "            |

5 ml of cell suspension in nutrient broth of lysogenic and nonlysogenic *S. marcescens* taken in a petridish was irradiated with a Philips U.V. lamp from a distance of 15 cm. Then the treated suspension was plated (0.1 ml) on *S. marcescens* as the indicator organism (1 ml of 24 hrs culture).

TABLE III

Pigment production by lysogenic and nonlysogenic strains of *S. marcescens*

| Expt. No. | Strain       | Wt. of the cell/10 ml | Pigment content/5 ml of cultures expressed as Klett-Reading at 540 mμ |
|-----------|--------------|-----------------------|---|
| 1         | Nonlysogenic | 0.0085                | 110   |
|           | Lysogenic    | 0.0085                | 185   |
| 2         | Nonlysogenic | 0.0085                | 110   |
|           | Lysogenic    | 0.0085                | 185   |
| 3         | Nonlysogenic | 0.0085                | 110   |
|           | Lysogenic    | 0.0085                | 185   |

50 ml of nutrient broth taken in a conical flask was inoculated with the strains of *S. marcescens* and incubated for 10 days at 30° C. An aliquot of 40 ml was then worked up for pigment production according to the method of Williams, Green and Rapport<sup>8</sup>. Another aliquot of 10 ml was used to get dry weight of cells,

The lysogenic strain was isolated from a turbid plaque obtained by plating on the nonlysogenic strain the temperate phage *kappa*. The isolate was tested for its phage resistance pattern (Table I), U.V. induction of lysis (Table II) and pigment production capacity (Table III). The pigment produced was also subjected to spectral analysis at neutral, acidic and alkaline pH using 3 ml of alcoholic extract and adding 0.3 ml of 1 N HCl or 1 N NaOH for acidic or alkaline pH.

Table I shows that the isolate resists lysis when the phage *kappa* was plated on it. On exposure to U.V. the isolate produces plaque (Table II) while the parent nonlysogenic strain fails to do so under similar conditions. Thus the isolate is a true lysogen and not a phage resistant mutant of *S. marcescens*.

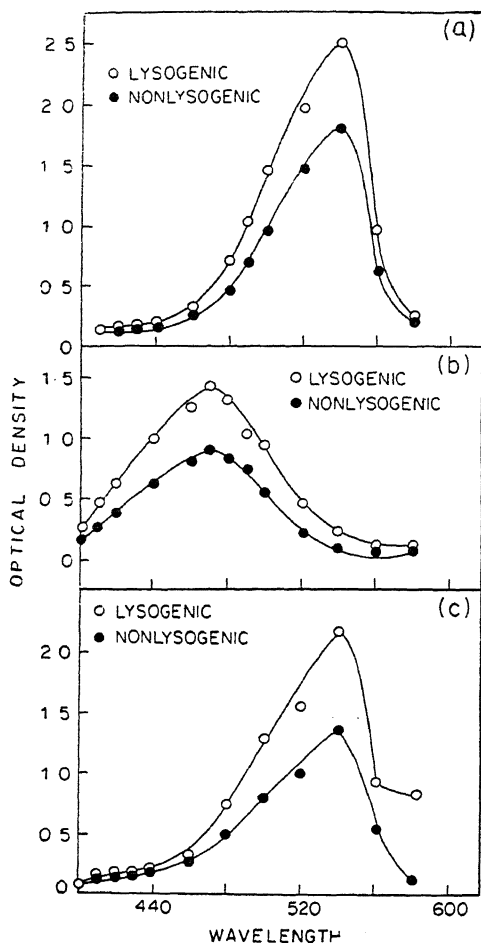


FIG. 1. Absorption spectrum in the visible range under acidic (a), alkaline (b) and neutral (c) condition of the pigment synthesised by lysogenic and nonlysogenic strain of *S. marcescens*.

Table III shows that pigment produced by the lysogen is almost double the amount produced by the nonlysogen. The increased pigment production is not due to cell mass since the cell mass for the lysogenic as for the nonlysogenic strain is nearly the same. Moreover Figs. 1 and 2 show that there is no appreciable changes in the spectral behaviour of pigments, which suggests that the nature of pigment synthesised by both lysogenic and nonlysogenic strains may be the same. Lysogenic conversion with respect to pigment production is thus indicated.

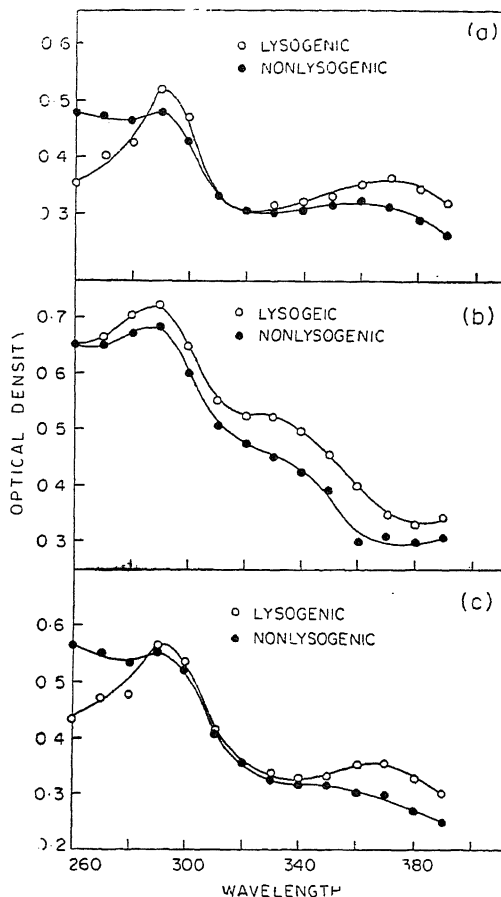


FIG. 2. U.V. absorption spectrum under acidic (a), alkaline (b) or neutral (c) condition of the pigment synthesised by lysogenic and nonlysogenic strain of *S. marcescens*.

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#### EFFECT OF GIBBERELIC ACID ON ALPHA AMYLASE ACTIVITY OF MUTAGEN-TREATED BARLEY SEEDS

GIBBERELIC acid ( $GA_3$ ) is known to enhance the alpha amylase activity in the endosperm of barley seeds and other grain crops. Ananthswamy, 1971 in wheat; Muller, 1971 in pea; Palge, 1960 in barley; Varner, 1964 in barley. However, it is not known if gibberellic acid will have any modifying effect on the alpha amylase activity of mutagen treated barley seeds. In the present investigation, the effect of gibberellic acid on seeds treated with mutagenic agents, viz., ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS) and gamma rays (Kr) was investigated.

Irradiation of barely, seeds, variety RS 6, was done in the Division of Genetics, Indian Agricultural Research Institute, Delhi. Treatment of seeds with EMS and MMS (Eastman Kodak Company, U.S.A.) was done by dissolving different concentrations of the mutagens in 0.02 M phosphate buffer at pH 6.9. Seeds were first soaked in different concentrations of EMS and MMS for 10 hours after which these were washed several times with distilled water and then placed in 1000 ppm gibberellic acid solution (Phylaxia, Budapest) for 8 hours.

Gibberellic acid (1000 ppm) was prepared by dissolving 0.1 gm of gibberellic acid in one ml absolute alcohol and volume made up to 100 ml with distilled water. Irradiated seeds were also treated similarly by soaking in distilled water for 12 hours, after which these were taken out and placed in gibberellic acid solution for 8 hours.

From each treatment, 0.5 gm of the seed was taken and ground in a pestle and mortar with 15 ml of 0.02 M phosphate buffer (pH 6.9) at 8° C and filtered through Whatman No. 1 filter-paper, following the method of Bernfield, 1960. One ml of this filtrate was made up to 25 ml with distilled water, which was incubated for three minutes at 20° C, after mixing with one ml of 1% starch. The enzyme reaction was interrupted by

the addition of 2 ml of 3-5-dinitrosalicylic acid and was heated for 5 minutes in boiling water and then cooled in running tap water. After the addition of 15 ml of distilled water, the optical density of the solution containing the brown reduction product was determined at 540 m $\mu$  using Spectronic-20. The blank is prepared in the same manner without adding enzyme preparation. A calibration curve established with maltose was used to convert the reading of optical density into milligrams of maltose.

The alpha amylase activity is expressed in terms of milligrams of maltose liberated in three minutes at 20° C by 1 ml of the enzyme solution of mutagen and gibberellic acid treated barley seeds.

Irradiated barley seeds with different dosage of gamma rays and then treated with gibberellic acid were analysed for alpha amylase activity. Twenty seeds were used for each treatment. In control, the seeds were treated with distilled water and gibberellic acid.

TABLE I  
Effect of gibberellic acid (1000 ppm) on alpha amylase activity of irradiated seeds

| Treatment                   | Enzyme Activity<br>(mg of maltose)<br>in (Kr) treated<br>seeds | Enzyme Activity<br>(mg of maltose)<br>in (Kr - Gibbe-<br>rellic acid)<br>treated seeds |
|-----------------------------|--|--|
| 20 Kr                       | 0.5  | 0.71   |
| 30 Kr                       | 0.43   | 0.55   |
| 40 Kr                       | 0.38   | 0.5  |
| 50 Kr                       | 0.29   | 0.38   |
| Control—water               | 0.58   | ..   |
| Control—gibberellic<br>acid | 0.82   | ..   |

In Table I, it is indicated that gibberellic acid increases alpha amylase activity in both irradiated and controlled barley seeds. However, with increased dosage of irradiation, there is a corresponding decrease in the enzyme activity.

In Table II, it is found that there is a reduction in the alpha amylase activity when barley seeds are treated with the mutagenic agents EMS. But when such seeds are treated with gibberellic acid ( $GA_3$ ) for 8 hours, the alpha amylase activity increases.

The effect of gibberellic acid on the recovery of the enzymatic activity of the irradiated barley seeds is 90% higher at 20 to 50 Kr. But it decreases as the Kr dosage increases. There is not much recovery of the enzyme activity in the case of EMS and MMS treated barley seeds. It is also seen that

TABLE II

Effect of gibberellic acid ( $GA_3$ ) on alpha amylase activity of EMS and MMS treated barley seeds

| Concentration %          | Enzyme Activity (mg of maltose) in MMS and EMS treated seeds | Enzyme Activity (mg of maltose) in $GA_3$ + MMS and EMS treated seeds |
|--------------------------|--|---|
| <b>MMS</b>               |  |   |
| 0.0125                   | 0.32   | 0.38  |
| 0.025                    | 0.29   | 0.33  |
| 0.05                     | 0.25   | 0.27  |
| 0.075                    | 0.22   | 0.23  |
| <b>EMS</b>               |  |   |
| 0.125                    | 0.37   | 0.45  |
| 0.25                     | 0.35   | 0.38  |
| 0.5                      | 0.29   | 0.35  |
| 0.75                     | 0.19   | 0.30  |
| Control—water            | 0.58   | ..  |
| Control—gibberellic acid | 0.82   | ..  |

the damage to the enzyme activity, caused by the irradiation of the seeds, is different with the EMS and MMS treated barley seeds. However, the possibility of a higher recovery of the enzyme activity in EMS and MMS treated barley seeds by increasing the amount of gibberellic acid ( $GA_3$ ) may exist and work is in progress.

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#### A NOTE ON THE ORIGIN OF LEPTYNITES

THE frequent occurrence of leptynites (garnetiferous quartzo-feldspathic granulites) and khondalites in association with the charnockite suite of rocks is interesting from the point of view of their origin. While khondalites have been proved to be definite metamorphosed pelites, opinions differ with regard to the premetamorphic origin of leptynites and there are arguments in favour of ortho, para, as

well as migmatitic origin for these rocks. While Holland<sup>1</sup> regarded leptynites as products of dynamo-metamorphosed charnockites, Subramanyam<sup>6</sup> regarded the leptynites of Madras area to be an intensely metamorphosed recrystallised and reconstituted facies of khondalites. On the other hand Viswanathan *et al.*<sup>8</sup>, Narayanaswamy<sup>4</sup> and Ramaswamy *et al.*<sup>5</sup> have argued in favour of migmatitic origin for these granulites.

Leake<sup>2</sup> has deduced certain critical diagnostic graphs which involve Niggli mg, C, al-alk and trace elements Ni and Cr, in order to discriminate between ortho and para amphibolites. Van de Kamp<sup>7</sup> used these diagrams to study the premetamorphic origin of metasediments in Haliburton, Madoc area, SE Ontario. The critical graphs are plot of Niggli mg against C, C against al-alk, trilinear 100 mg, C, al-alk where  $mg + C + (al-alk) = 100$ , mg against Cr, and mg against Ni.

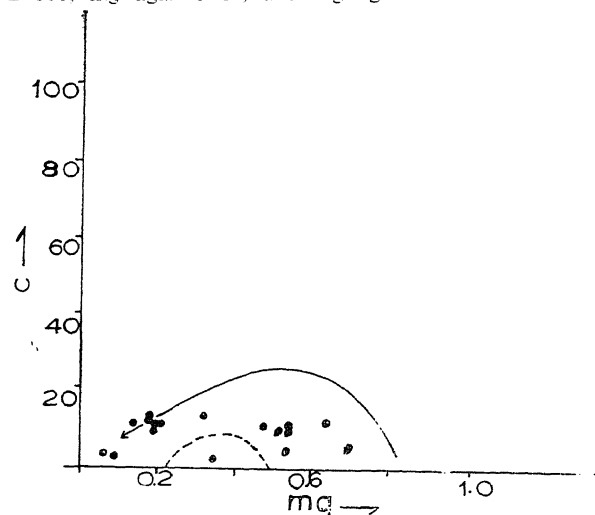


FIG. 1. Niggli C against mg plot. The dashed curve outlines the pelite area and the thick line indicates the igneous trend of variation—from Van de Kamp<sup>7</sup>. The plot of leptynites spread between igneous line and field of pelites.

The behaviour of these elements is unusually sensitive and diagnostic of the rocks. When they are plotted for a range of igneous and sedimentary rocks they fall in markedly different fields and show definite trends of variation. The reliability of the criteria is demonstrated by Leake<sup>2-3</sup>. The authors in the present paper have attempted to extend these criteria in order to understand the premetamorphic origin of the leptynites. Eighteen analyses of leptynites are plotted. Twelve of them were collected from the literature<sup>5-9</sup> (Puri Dist.—1, Madras—4, Capecomorin—3, Palani—1, and Guntur Dist.—3). Six fresh analyses were carried out, three



each from Madras and Niligiri areas, for major elements and traces Ni and Cr. Figures 1, 2 and 3 are reproduced from Van de Kamp<sup>7</sup> to show different fields of igneous and sedimentary rocks and trend of their mixtures. The analyses of leptynites are plotted on these diagrams.

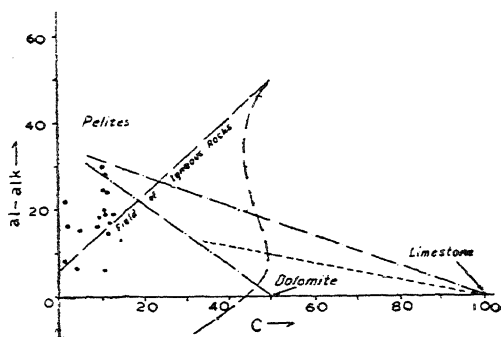


FIG. 2. Plot of C against al-alk. The differences between the igneous trend and those for pelite-carbonate mixtures are shown—from Van de Kamp<sup>7</sup>. The leptynite plots trend between low C and al-alk field and field of pelites.

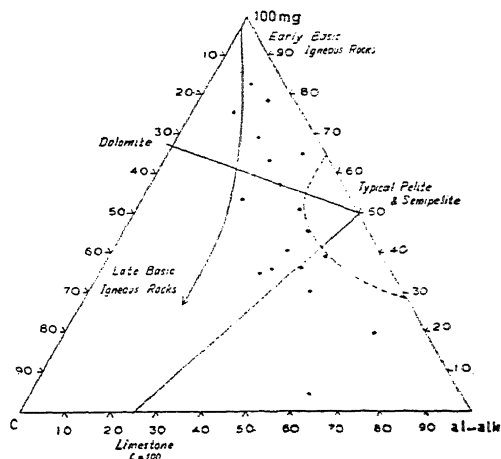


FIG. 3. Triangular plot C, al-alk, and 100 mg indicating the igneous trend obtained for Karoo dolerites and those to be expected for pelite-carbonate mixtures. Reproduced from Van de Kamp<sup>7</sup>. The leptynite plots lie between the igneous line and field of pelites and semipelites.

Figure 1 is the plot of Niggli mg against C. The thick curve indicates the trend of igneous differentiation for Karoo dolerites<sup>2</sup>. The dotted line encloses the field of pelites and semipelites. The plots of leptynites (except one) scatter between the pelite field and the line of igneous differentiation. The same situation is observed in Fig. 3, where Niggli mg, C and (al-alk) are plotted on a trilinear diagram where once again they are distributed between the pelite field and the igneous line. Figure 2 is more

critical where the slope of the leptynite plot shows a negative correlation and trends against the line of igneous differentiation. It extends between the low C and al-alk region and the field of pelites. This suggests that the leptynites were either original pelites mixed with psammites or arkoses, or they were original pelites into which there was feldspathisation with the influx of alkalis. Figures 1 and 3 are not very conclusive but they certainly do not indicate pure igneous or pure pelitic parentage to these granulites. The plot of Niggli mg against Cr and Ni is critical to discriminate whether the premetamorphic rock is a mixture of sediments of different compositions or a mixture of igneous and sedimentary series.

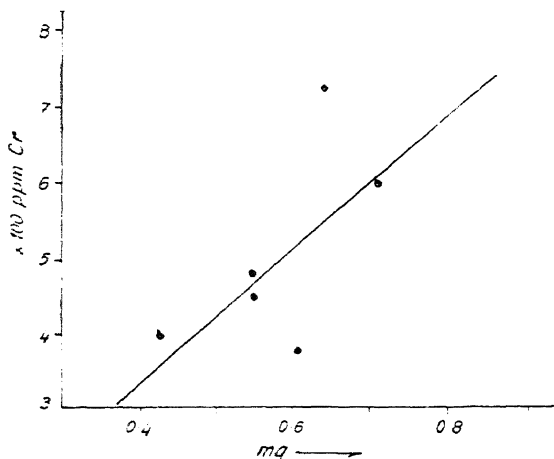


FIG. 4. Plot of Cr against mg for Madras and Niligiri leptynites. Note the positive correlation.

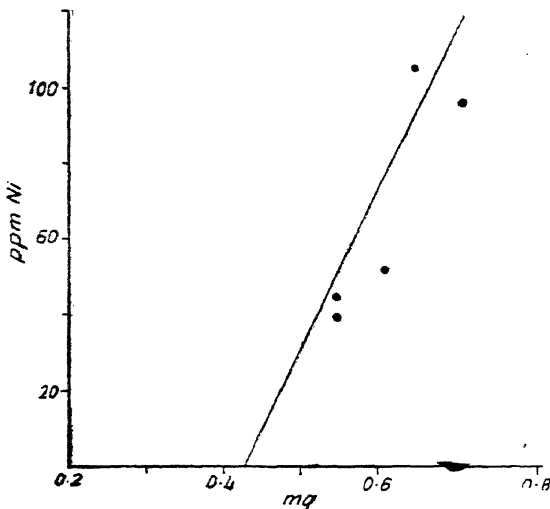


FIG. 5. Plot of Ni against mg for Madras and Niligiri leptynites. Note the positive correlation.

If the leptynites are formed by the metamorphism of the original pelites mixed with psammities or arkoses, the plot mg against Cr and Ni will give a negative correlation. On the contrary if they were original pelites into which were showered volcanic rocks, the plots would show a positive correlation (Leake, personal communication).

Nickel and chromium determined for six Nilgiri and Madras leptynites when plotted against Niggli mg show positive correlation (Figs. 4 and 5).

The authors therefore suggest that these leptynites have a hybrid premetamorphic origin. They were originally pelites which suffered igneous activity with the influx of alkalies and also alumina prior to metamorphism which resulted in the present garnetiferous quartzofeldspathic assemblage.

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#### SEXUAL DIMORPHISM IN THE CLOACA OF *CALOTES VERSICOLOR* (DAUD)

WHILE working out the biology of *Calotes versicolor* we had opportunity of examining large number of lizards. Though the matured males are brilliantly coloured red yellow during breeding season the immature and young specimens give considerable difficulty in distinguishing sex externally as lizards look alike in their features. Smith (1935) states that there is still much to be learnt with regard to sexual dimorphism in lizards and it is important, therefore, to be able to sex a specimen accurately.

A closer observation of *C. versicolor* revealed that the cloacal structure showed sexual dimorphism. The dimorphic characters are diagrammatically represented in Fig. 1. A slit-like transverse

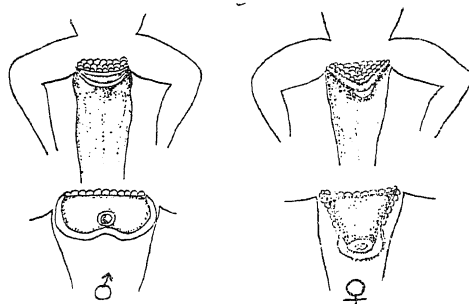


FIG. 1. Diagrammatic representation of cloaca showing dimorphic characters of *Calotes versicolor*.

cloacal aperture at the base of the tail occurs on the ventral surface. In natural state the cloacal aperture in male has two lips—an upper and a lower—covered with minute scales. When the tail is pulled down to expose internal cloacal aperture the exposed cloacal area gives a semicircular appearance with circular cloacal aperture lying in the centre of the circumference, while in female the cloaca has an external triangular flap covering the slit-like cloacal aperture and bears a nodular projection in the lower lip. The exposed cloacal area appears triangular and the internal cloacal aperture lies at the apex of the triangle. Blank (1967) described superficial sexual dimorphic characters in *Zonosaurus maximus* of Madagascar where in male there is an oval and in female a triangular large scale covering cloaca. These dimorphic characters are characteristic features of *Calotes* and can be reliably used for distinguishing the sexes of the lizards for using them for experimental purposes.

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#### OCCURRENCE OF *OLPIDIUM BRASSICAE* IN INDIA

WORONIN (1878)<sup>2,5</sup>, while working with the club root disease of cabbage, observed a chytridiaceous fungus parasitizing the roots of crucifers and named it as *Chytridium brassicae* Woronin. The fungus was later transferred to the genus *Olpidium* (Braun) Rabenh. by Dangeard (1886)<sup>2,5</sup>. Relatively few

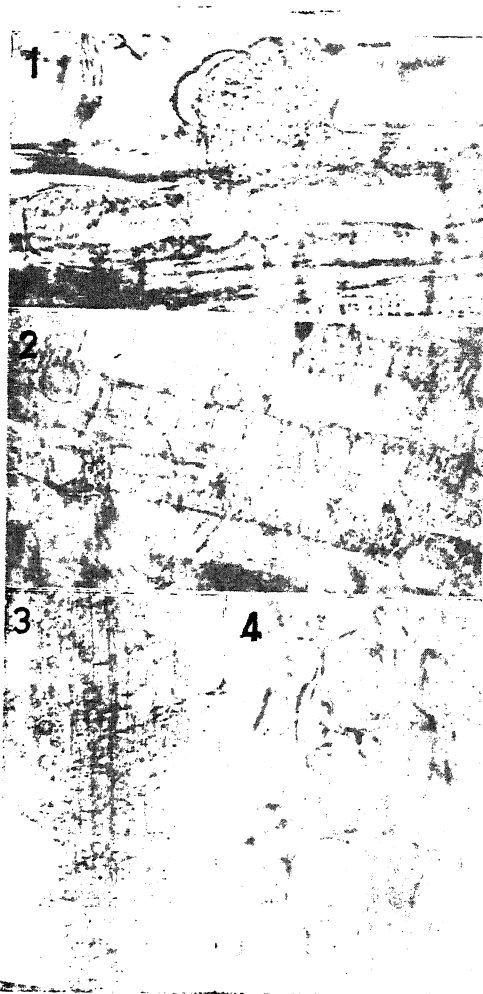
species of the genus *Olpidium* are reported parasitic in the roots of crop plants from the world<sup>1-5</sup>. Among these *Olpidium brassicae* has been known widely distributed and its parasitism reported in the seedling roots of several phanerogams.

During a survey of the root diseases of cruciferous crops, a chytridiaceous parasite was consistently encountered in the young roots of cabbage (*Brassica oleracea* L. var. *capitata*) and cauliflower (*Brassica oleracea* L. var. *botrytis*) seedlings in the low-lying, ill-drained heavy soil fields (pH 7 to 8.5) of Varanasi, U.P., during December and January. Diseased plants did not show any marked symptoms and incipiently infected seedlings developed normally. The infected roots looked apparently healthy, but in severe infections few rootlets and secondary roots showed pale reddish brown discoloration at the tips and slight unthriftness or sickness was the only sign of the disease noticed in the plants in the field and in pot cultures. Cabbage and cauliflower seeds sown in infested soil germinated normally and the seedlings developed with no external disease symptoms, but microscopic examination of the roots revealed abundant development of intracellular zoosporangia and resting spores of the pathogen.

The thalli developed in the first and third or fourth layers of the epiblema and were most abundant in the outermost layer. They were holocarpic, mostly cylindrical, thin-walled, with densely granular protoplasmic contents, variable in shape, measuring  $50-152.5 \times 14-23 \mu$  (Fig. 1). The thalli segmented into merons in a row, which became transformed into spherical to ellipsoidal, thin-walled zoosporangia singly or in a row in the cortical cells, being abundant in the outer layers (Fig. 2). Very few zoosporangia developed in the inner cell layers. They measured  $10-50.8 \times 10-35.6 \mu$ . Their walls were smooth and highly permeable to stains. Dense protoplasmic contents in maturing zoosporangia cleaved into uniflagellate zoospores, which escaped through the canal of the exit tube extending to the exterior or sometimes opening within the host tissues. The zoospores were numerous, spherical to pyriform,  $5.0-6.4 \mu$  in diam., bearing a single posterior flagellum,  $19.0-29.2 \mu$  in length.

Intracellular resting spores were formed abundantly in the outer cell layers often at the base of or within the root hairs and frequently in the deeper (3rd or 4th) layers (Fig. 3). They were thick-walled, hyaline and oval to globose in shape. The exospore was thick and warty. The endospore was thin and smooth. The resting spores measured  $9.2-38 \times 11.5-26.6 \mu$  (Fig. 4).

Morphology and development of sporangia, zoospores and resting sporangia of the pathogen indicated its identity to *Olpidium brassicae* (Wor.) Dangeard, to which it is referred. *Olpidium uredinis* (Lager.). Fischer and *O. indicum* Turner respectively parasitic in the urediospores of *Hemileia canthii* Berk. and Br. and in the filaments of *Oedogonium* sp. are the other species hitherto reported to occur in India<sup>6,7</sup>. The occurrence of *Olpidium brassicae* in the roots of cabbage and cauliflower constitutes the first record for India (IMI 181965, HClO 31782).



Figs. 1-4. Fig. 1. Thallus of *Olpidium brassicae* within cortical cells of cabbage root. Fig. 2. Rows of thin-walled zoosporangia in the cortical cells. Fig. 3. Portion of rootlet showing abundance of resting spores. Fig. 4. Resting spores showing typical warty surface.

(Figs. 1, 2, 4,  $\times 530$ ; Fig. 3,  $\times 35$ .)

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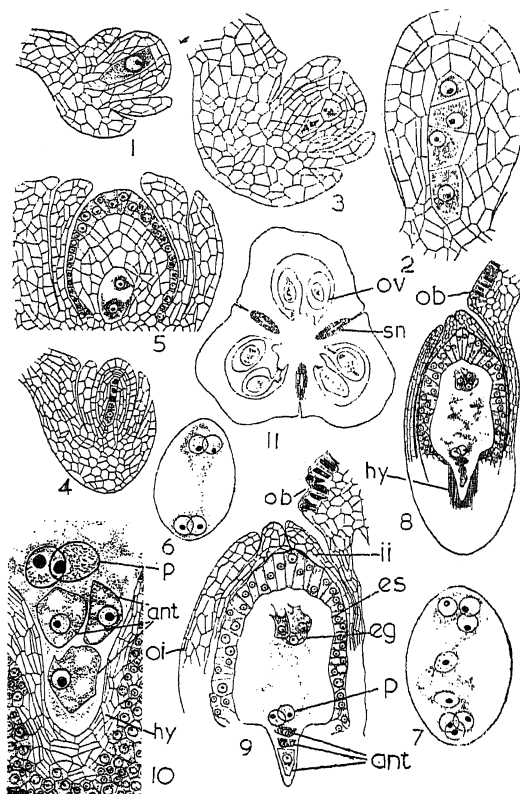
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#### EMBRYO SAC DEVELOPMENT IN *SCILLA PERUVIANA* L.

THE two genera *Scilla* and *Endymion* were merged into a single genus *Scilla* for some time. Chouard (1931, 1934), however, restored *Endymion* to its former status so that the old genus *Scilla* of Linnaeus is now subdivided into *Urgenia* (Steinh.) Baker; *Scilla* (L.) Baker Emend. Chouard and *Endymion* (Dum.) Chouard. Two species of *Endymion*, *E. hispanicus* (= *Scilla hispanica*) and *E. non-scriptus* (= *Scilla non-scripta*) show a bisporic 8-nucleate embryo sac developed from the upper dyad cell, while the lower one develops upto the 4-nucleate stage finally forming the antipode. In the embryologically known species of *Scilla*, *S. indica* (Govindappa and Sheriff, 1951; Sulbha, 1954), *S. hyacinthina* (Sulbha, 1954), *S. autumnalis* (Battaglia, 1958) and *S. pratensis* (Battaglia and Feeley, 1959) and *Urgenia indica* (Capoor, 1937) a Polygonum type of embryo sac development has been reported. Battaglia and Feeley (1959) expressed the opinion that species of *Scilla* follow the Polygonum type of embryo sac development and those of *Endymion* conform to the special bisporic *Endymion* type. The present embryological study was endeavoured to trace the embryo sac development in *Scilla peruviana* L., a Mediterranean species, and to examine its bearing on its systematic position.

The tricarpellary ovary is superior, syncarpous and trilobular. Septal nectaries are present in the ovary and their pockets are lined with glandular cells (Fig. 11). A point of interest is the occurrence of abundant raphides in the crystal sacs of all the floral parts. The numerous anatropous,

bitegmal and crassinucellar ovules are collaterally borne on axile placentae (Fig. 11).



Figs. 1-11. Fig. 1. L.s. young ovule showing megaspore mother cell and the parietal cell,  $\times 30$ . Fig. 2. L.s. nucellus showing linear tetrad of megaspores,  $\times 50$ . Fig. 3. L.s. ovule showing dyad cells under division,  $\times 30$ . Fig. 4. L.s. ovule showing the chalazal megaspore functioning and other degenerating,  $\times 30$ . Fig. 5. L.s. ovule showing 2-nucleate embryo sac. Note the vacuolated and radially enlarged nucellar epidermal cells,  $\times 50$ . Figs. 6, 7. Four and eight-nucleate embryo sacs,  $\times 50$ . Fig. 8. L.s. ovule showing the mature embryo sac, hypostase, enlarged nucellar epidermal cells and funicular obturator,  $\times 20$ . Fig. 9. L.s. part of ovule showing mature embryo sac, funicular obturator and the radially enlarged nucellar epidermal cells,  $\times 25$ . Fig. 10. Chalazal part of the ovule showing hypostase, hypertrophied antipodals and polar nuclei,  $\times 50$ . Fig. 11. C.s. Ovary. Note septal nectary,  $\times 3$ . (ant, antipodals; eg, egg apparatus; es, embryo sac; ii, inner integument; ob, funicular obturator; oi, outer integument; ov, ovules; hy, hypostase; p, polars; sn, septal nectary.)

A single hypodermal archesporial cell differentiates about the time the integumentary primordia begin to make their appearance. The archesporial cell cuts off a parietal cell (Fig. 1) which divides anticlinally and the derivatives thereupon divide

periodically. The megaspore mother cell undergoes the two meiotic divisions to procreate a tetrad of megaspores of which the chalazal only functions (Fig. 4). The tetrad of megaspores is either linear (Fig. 2) or T-shaped (Fig. 3). The nucleus of the chalazal megaspore undergoes the three free nuclear divisions resulting in an 8-nucleate embryo sac (Figs. 5, 6, 7), while the three micropylar ones degenerate (Fig. 4). The embryo sac development, therefore, is of the Polygonum type.

During the development of the female gametophyte there is a considerable enlargement leading to the destruction and absorption of the cells of the nucellus all around excepting at narrower chalazal part of the embryo sac (Figs. 8, 9). The persisting nucellar epidermal cells enlarge radially and the cells become highly vacuolated. The mature embryo sac shows the 3-celled egg apparatus, the two polar nuclei, which fuse in the vicinity of the antipodal cells resulting in the secondary nucleus, and the three antipodal cells which become hypertrophied and occupy the narrow chalazal part of the embryo sac (Figs. 8, 9, 10). A hypostase is discernible at the organised embryo sac stage (Fig. 8). In some preparations one or two antipodal cells were found to degenerate (Figs. 8, 9).

About the time the embryo sac matures a funicular obturator was found to be differentiating (Figs. 8, 9), a feature which does not seem to have been recorded for embryologically known species of *Scilla*.

From the evidence so far available, it is obvious, that the type of embryo sac development seems to favour the treatment of *Scilla* and *Endymion* as distinct genera.

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## TOTAL SILICA AND BROWN SPOT DISEASE DEVELOPMENT OF RICE UNDER VARYING LEVELS OF NITROGEN

PRESENCE of silica in increased quantity in the leaves of wheat and rice plants conferred resistance to powdery mildew, blast and brown spot disease<sup>1,2</sup>. No data are available on the susceptibility of the rice plants to brown spot disease incited by *Helminthosporium oryzae* Breda de-Haan (under different doses of nitrogenous fertilization) and their silicon status of the leaves. The present investigation deals with this aspect.

Rice seedlings were raised in plastic pots (30 cm diameter) filled with quartz sand nourished with Hoagland solution which was adjusted for the various levels of nitrogen. Nitrogen was applied in the form of ammonium sulphate. There were 7 treatments, i.e., 0–60 Kg N/ha at 10 Kg interval with 3 replications. Patnai-23 and latisail for winter and latisail and CB-1 were chosen for summer season. Thirty days, 75 days and 90 days old plants were considered for seedling, tillering and preflowering stages of growth. For inoculation, H 39 strain of *H. oryzae* having 7,000 viable conidia/ml water was sprayed mixing a few drops of trixon-x as adhesive. The pots were flooded with sterile distilled water and kept under incubation for 96 hours. Infection value was calculated by collecting 25 leaves for each grade after Padmanabhan<sup>4</sup>. The pots were stored in a cool, dark place for 24 hr before collecting leaf samples for total silica analysis; just after recording the infection values. Total silica was analysed by standard method<sup>5</sup>.

For finding out the correlation between incidence of brown spot and percentage of total silica in rice leaf blades, statistical analysis was worked out<sup>6</sup> and the correlation value  $r$  together with degrees of freedom are presented in Tables I and II. The percentage of total silica for summer rice varieties, i.e., Latisail and CB-1 (Figs. 1, 2 and 3) was negatively correlated with the brown spot disease development. Statistically significant relationships for autumn and winter rice varieties (Tables I and II) were obtained between the silicon status of the host plant and intensity of infection of brown spot disease incited by *H. oryzae*. Higher concentration of total silica was recorded at the post-tillering stage in all the seasons while the disease intensity was minimum and the lowest was noted at the seedling stage which were attended with highest infection values. Similar observations were recorded in all the seasons. Increased amount of silica was noted in the rice leaves grown in summer and lowest in winter season whereas autumn rice showed the intermediate reaction. An interesting

TABLE I

Data on the percentage of total silica and infection values of two vars. of autumn rice at three different stages of growth

| Vars.   | N level<br>Kg/ha | Seedling stage      |                    | Post-tillering stage |                    | Preflowering stage  |                    |
|---------|------------------|---------------------|--------------------|----------------------|--------------------|---------------------|--------------------|
|         |                  | Total silica<br>(%) | Infection<br>value | Total silica<br>(%)  | Infection<br>value | Total silica<br>(%) | Infection<br>value |
| Dular   | 0                | 3.39                | 60.8               | 6.63                 | 35.0               | 5.38                | 48.8               |
|         | 10               | 3.86                | 50.6               | 7.47                 | 32.0               | 6.70                | 46.2               |
|         | 20               | 4.65                | 48.0               | 9.30                 | 22.6               | 8.36                | 41.8               |
|         | 30               | 4.64                | 46.6               | 9.32                 | 23.2               | 8.34                | 40.4               |
|         | 40               | 4.64                | 46.8               | 9.30                 | 23.8               | 8.32                | 40.0               |
|         | 50               | 3.61                | 56.4               | 5.57                 | 40.4               | 4.40                | 55.2               |
|         | 60               | 2.14                | 84.0               | 4.67                 | 68.0               | 3.30                | 62.0               |
| Dhairal | 0                | 3.03                | 62.4               | 6.10                 | 38.0               | 4.95                | 53.0               |
|         | 10               | 3.05                | 58.8               | 7.06                 | 36.0               | 6.00                | 39.4               |
|         | 20               | 4.42                | 52.8               | 9.01                 | 25.4               | 7.12                | 32.8               |
|         | 30               | 4.32                | 50.2               | 9.11                 | 25.6               | 7.16                | 32.4               |
|         | 40               | 4.38                | 50.0               | 9.15                 | 25.6               | 7.18                | 32.0               |
|         | 50               | 2.48                | 82.6               | 5.42                 | 43.0               | 3.42                | 58.2               |
|         | 60               | 2.08                | 89.6               | 3.20                 | 53.0               | 2.93                | 86.0               |

Correlation between total silica and infection value.

|         |          |          |         |
|---------|----------|----------|---------|
| D.F.    | 12       | 12       | 12      |
| r value | -0.95*** | -0.88*** | 0.95*** |

TABLE II

Data on the percentage of total silica and infection values of two rice vars. of winter rice at three different stages of growth

| Vars.     | N level<br>Kg/ha | Seedling stage      |                    | Post-tillering stage |                    | Preflowering stage  |                    |
|-----------|------------------|---------------------|--------------------|----------------------|--------------------|---------------------|--------------------|
|           |                  | Total silica<br>(%) | Infection<br>value | Total silica<br>(%)  | Infection<br>value | Total silica<br>(%) | Infection<br>value |
| Patnai 23 | 0                | 1.95                | 88.0               | 5.38                 | 48.8               | 4.22                | 53.6               |
|           | 10               | 2.82                | 80.0               | 5.62                 | 43.8               | 4.98                | 50.4               |
|           | 20               | 3.53                | 50.4               | 8.18                 | 34.4               | 7.22                | 32.2               |
|           | 30               | 3.53                | 50.6               | 8.16                 | 28.4               | 7.24                | 32.0               |
|           | 40               | 2.51                | 88.0               | 8.18                 | 27.2               | 6.14                | 40.1               |
|           | 50               | 2.60                | 84.2               | 5.39                 | 40.4               | 4.26                | 49.8               |
|           | 60               | 1.25                | 91.6               | 4.60                 | 50.4               | 3.11                | 59.2               |
| Latisail  | 0                | 1.65                | 90.8               | 5.02                 | 41.0               | 3.81                | 58.0               |
|           | 10               | 2.25                | 83.2               | 5.17                 | 45.0               | 4.53                | 53.0               |
|           | 20               | 3.12                | 52.2               | 7.77                 | 36.6               | 6.82                | 37.3               |
|           | 30               | 3.11                | 52.8               | 7.65                 | 33.2               | 6.92                | 35.2               |
|           | 40               | 3.14                | 56.2               | 7.72                 | 34.9               | 6.03                | 36.0               |
|           | 50               | 2.17                | 88.0               | 5.16                 | 46.0               | 3.17                | 51.6               |
|           | 60               | 1.06                | 95.4               | 3.01                 | 74.2               | 2.92                | 82.0               |

Correlation between total silica and infection value

|         |          |          |          |
|---------|----------|----------|----------|
| D.F.    | 12       | 12       | 12       |
| r value | -0.97*** | -0.95*** | -0.98*** |

result (Figs. 1, 2 and 3) was observed in case of different doses of nitrogen. i.e., higher percentage of total silica at 20–40 kg N/ha level in all the seasons and at all the stages of the growth period which was inversely correlated with brown spot disease development. With excess of nitrogen level, i.e., 50–60 kg N/ha the disease intensity also increased and the silicon status decreased and the same was true with deficient level of nitrogen, i.e., 0–10 kg N/ha. No remarkable difference could be observed between the varieties in a particular season. Tasugi and Yoshida<sup>7</sup> explained about decreased silicon uptake of graminaceous plants

under excess nitrogen nutrition. But the present investigation could not offer any explanation about the decreased silicon content in rice leaves under deficient nitrogen level.

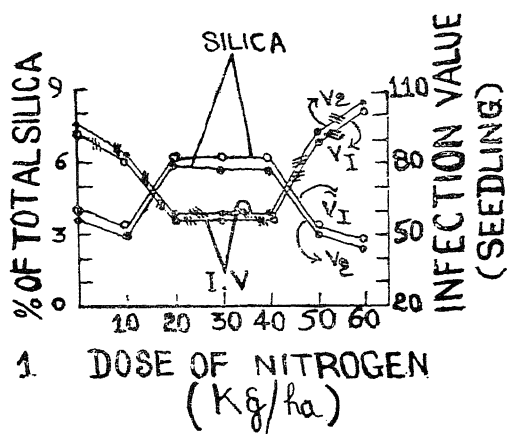


FIG. 1. Relationship of total silica to the incidence of brown spot disease of summer rice at 0–60 Kg N/ha level at seedling stage  $V_1$  = Latisail;  $V_2$  = CB-1.

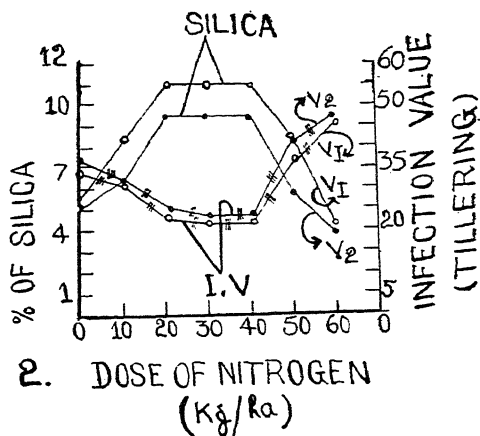


FIG. 2. Relationship of total silica to the incidence of brown spot disease of summer rice at 0–60 Kg N/ha level at tillering stage.  $V_1$  = Latisail,  $V_2$  = CB-1.

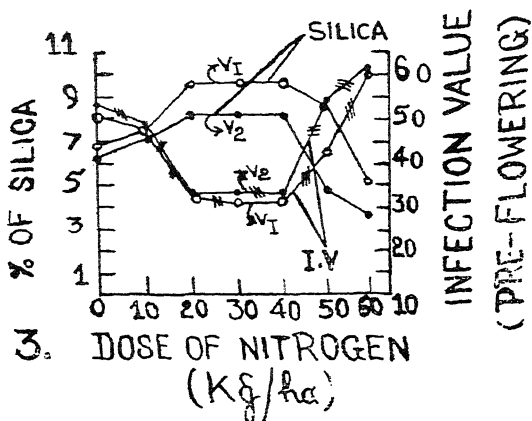


FIG. 3. Relationship of total silica to the incidence of brown spot disease of summer rice at 0–60 Kg N/ha level at pre-flowering stage  $V_1$  = Latisail,  $V_2$  = CB-1.

Thus it may be concluded that silicon content of rice leaves and brown spot disease incidence are negatively correlated. Lower amount of silica in rice leaves stand for susceptibility to brown spot disease. Balanced nitrogen nutrition might be an important criterion for increasing the silicon status of rice leaves and making the rice crops less susceptible to brown spot disease.

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**MERREMIA PELTATA (LINN.) MERR. (CONVOLVULACEAE)—A NEW RECORD TO INDIAN FLORA FROM GREAT NICOBAR ISLAND**

DURING a Joint Scientific Expedition to the Great Nicobar Island in 1966, an interesting member of Convolvulaceae was collected which on critical studies turned out to be *Merremia peltata* (Linn.) Merr., a species hitherto unrecorded from India. The plant is distributed in Malesia, Philippines, New Guinea, Madagascar, Mascarenes, Sechelles, Tropical Australia and Polynesia. The occurrence of this taxon in Great Nicobar Island is therefore not only a new record for India but represents an extension of its range of distribution up to the Nicobar Island. A detailed description of the taxon with an illustration is presented in this paper.

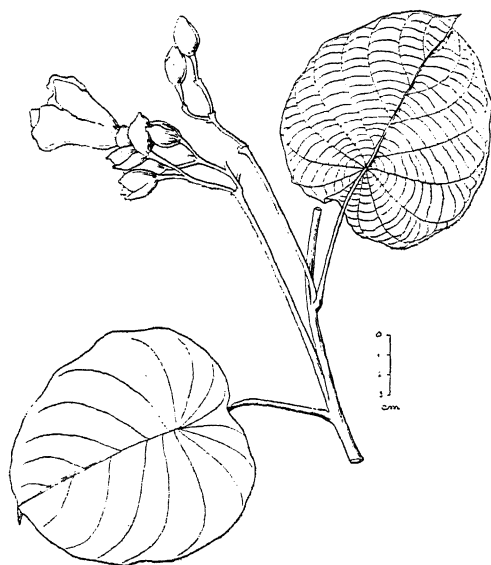


FIG. 1. *Merremia peltata* (Linn.) Merr. A branch with flowers.

*Merremia peltata* (Linn.) Merr. Interpr. Rumph., *Herb. Amb.* 441, 1917; Ooststr. in *Blumea* 3: 352, 1939, et van Steenis in *Fl. Males.* (Ser. 1) 4: 452, 1973; Backer et Bakhuizen van Den Brink, *Fl. Java* 2: 489, 1965. *Convolvulus peltatus* Linn. *Sp. Pl.* 1194, 1753. *Ipomoea nymphaeifolia* Bl. *Bijdr.* 719, 1825. *I. peltata* Choisy, *Mem. Soc. Phys. Geneve* 6: 452, 1833. *Merremia nymphaeifolia* Hallier, *Verslag. Buitenz.* 127, 1895; Ridley, *Fl. Mal.* 2: 458, 1923.

Twining. Leaves ovate-cordate to semi-orbicular 12–14 × 11.5–14.0 cm, alternate, long petiolate, distinctly peltate, entire, shortly apiculate at apex, glabrous; veins 8–9 pairs, reddish on the abaxial

side, radiating; petiole 7–12 cm long, glabrous. Flowers 2–4 in axillary cymes, yellow, 6–7 cm long; bracteate, bracteolate; pedicel 1.0–1.8 cm long. Sepals 5, free, coriaceous, 1.5–2.0 × 1.0–1.2 cm, greenish, imbricate, glabrous. Petals 5, united, bright yellow, glabrous; corolla tube funnel-shaped, 5 × 4 cm. Stamens 5, epipetalous; filaments dilated, hairy at base; anthers spirally twisted, hairy. Carpels 2, united, glabrous. Capsules subglobose, 4-loculed, 4-valved; seed 4, densely brown tomentose.

*Specimen examined*: Galathea river bank, Great Nicobar Island, 24–3–1966—*Thothathri and Banerjee* 11497 (CAL). A handsome twiner growing along river beds.

*Distribution*: Malesia, Philippines, New Guinea, Madagascar, Mascarenes, Sechelles and Tropical Australia.

Botanical Survey of India,  
Calcutta,  
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**INVESTIGATIONS ON A MOSAIC DISEASE OF CAPE GOOSEBERRY**

CAPE gooseberry (*Physalis peruviana* L.) is one of the commercially grown fruit crops in some parts of India. During 1973, a mosaic disease was observed on *Physalis peruviana* in the germplasm collection of *Physalis* spp. at the Indian Institute of Horticultural Research, Hessaraghatta. The characteristic feature of the disease is severe mosaic mottling of the leaves. The leaves are reduced in size and very often exhibited symptoms of malformation and distortion (Fig. 1). The affected plants are stunted in growth. The number of flowers and fruits per plant was found to be less when compared to healthy plants. Investigations were therefore undertaken to identify the causal virus and the results obtained are presented in this communication.

Under artificial inoculation, the virus was readily transmissible by sap to the herbaceous hosts but restricted in its host range to the plants belonging to families Amarantaceae, Chenopodiaceae and Solanaceae. It produced mosaic mottling symptoms on *Nicotiana debnyii* L., *N. plumbaginifolia* L., *N. rustica* L., *Physalis angulata* L., *P. ixocarpa* Brot., *P. minima*, *P. peruviana* L., *P. floridana* Rydb., *Nicandra physaloides* (L.) Pers., and *Petunia hybrida* Vilm. Chlorotic local lesions were observed on *N. tabacum* L. var. Xanthi and on *Chenopodium quinoa* Willd. Necrotic local lesions followed by systemic necrosis was observed on *N. tabacum* L. var. W.B., while necrotic local lesions alone were produced on inoculated leaves of *Chenopodium amaranticolor*



Coste and Reyn., *C. album* L., *C. ambrosioides* L.,  
*C. murale* L. and *Gomphrena globosa* L.

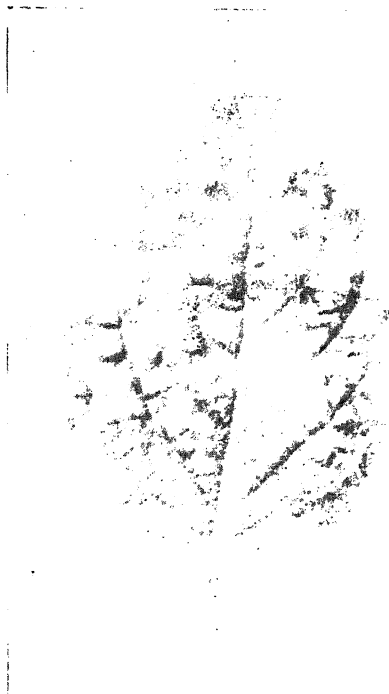


FIG. 1. Gooseberry leaf showing mosaic and distortion symptoms.

Out of four species of aphids tested, viz., *Myzus persicae* Sulz., *Aphis gossypii* Glov., *A. craccivora* Koch., and *Macrosiphum sonchi* L., only *M. persicae* transmitted the virus successfully. The aphids were given one hour pre-acquisition fasting and 15 minutes of acquisition feeding on diseased plants. After acquisition feeding the viruliferous aphids were allowed to feed on the test plants overnight and then they were killed by 0.02% parathion spray.

The gooseberry mosaic virus (GMV) inoculum (sap) could tolerate heating at 55° C. for 10 minutes, but not at 60° C. The virus was infective at a dilution of 1 : 1,000 but not at 1 : 10,000. The longevity *in vitro* of the virus in crude sap was found to be 4 days at room temperature (28–30° C) but it increased up to 10 days when the crude sap was stored at 5° C.

The virus under study was tested with different antisera, viz., Cucumber mosaic virus (CMV), Tobacco ring spot virus (TRSV), Tobacco etch virus (TEV), Potato virus Y (PVY), Potato virus X (PVX), Tobacco mosaic virus (TMV) and egg plant mosaic virus (EMV). However, no positive reaction was observed with any one of the antisera tested indicating that it is not related to them.

Capoor and Sharma (1965)<sup>1</sup> reported a mosaic disease of Cape gooseberry caused by TMV. The virus under the present study did not produce local lesions on *N. glauca* and it also showed negative reaction with TMV antisera. Therefore it cannot be TMV or its strains. Since the present virus did not induce any symptoms on any of the cucurbitaceous hosts, the possible relationships with CMV has been eliminated. This was also confirmed by serological tests. Chamberlain (1939)<sup>2</sup>, and Nariani and Sharma (1971)<sup>3</sup> reported a mosaic disease of Cape gooseberry caused by CMV (*Cucumis virus 1*). Moline and Fries (1972)<sup>4</sup> isolated a virus from *Physalis* species which was serologically related to Belladonna mottle virus (BMV) but the virus, reported herein, does not resemble in its physical properties and host range with BMV. Horvath (1970)<sup>5</sup> reported *P. peruviana* as a symptomless carrier of potato viruses X and Y. Based on the physical properties, host range, vector transmission and serological tests, the virus under study has tentatively been identified as a new virus causing mosaic disease of Cape gooseberry in India.

The authors are highly grateful to Dr. G. S. Randhawa for providing facilities and encouragement. Thanks are also due to Dr. C. P. A. Iyer and to Mr. M. D. Subramaniam, for supplying seeds of different *Physalis* species.

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#### **BALANOPHORA ABBREVIATA BLUME IN KARNATAKA**

THE curious root parasite *Balanophora abbreviata*, Balanophoraceae, was first collected in Java and described by Blume<sup>1</sup> in 1827. It has been subsequently recorded in Africa, Madagascar, Indo-China, Malaysia and the Pacific Islands. There is no mention of this species in any of the Indian Floras.

The authors have collected this taxon on the eastern bank of the Cauvery river near Ranganathittu, nine miles away from Mysore City, for the last five successive years, between July and October,

Since the species has a considerable range of distribution and recorded variability, an account of the observations made on the collected material is given below.

The fleshy achlorophyllous holoparasite is characterized by its distichous scale leaves and bisexual inflorescences with zygomorphic male flowers, located below the female part (Fig. 1). The plants



FIG. 1. *Balanophora abbreviata* Blume, adult plant with host roots.

grow in abundance, coming up like mushrooms, on the roots of *Acacia suma* Buch., Ham. and *Pithecolobium dulce* Benth. in sandy soil rich in humus well under shade. It is an annual appearing as small whitish or cream coloured underground nodules on newly produced young host roots soon after the first showers of the Southwest Monsoon. The nodules gradually develop into larger many-lobed tubers. The surface of the tuber is finely granular either with or without stellate warts. From each lobe bursts open a monoecious flowering scape bearing 4-6 distichous scale leaves, 20-40 almost sessile male flowers and innumerable female flowers. The bracts of the male flowers are small and needle-like when present. The tepals are usually four in number. The synandrium is laterally extended and consists of 15-25 anther loculi. The pollen grains are spherical and possess a finely spinulate exine bearing 8-12 circular pores. The female flowers are aggregated on an ovoid terminal capitulum. The spadiceles are long with an upper obconical part and a narrow lower region. The cuticular ridges of spadicele epidermis are conspicuous and lamellated. The archegonia-like pistils representing the female flowers are located both on the main

axis of the inflorescence and at the bases of the spadiceles.

During post-fertilization stages the male flowers wither, while the female flower bearing region enlarges considerably and acquires a dark gray colour. At the end of the growing season, by October, the entire plant body collapses and disappears leaving grain-like minute fruits, simulating the sand grains, to germinate and produce the next crop of parasite on young newly produced host rootlets in the succeeding season.

The taxon described by Haines<sup>2</sup> in his Botany of Bihar and Orissa as *B. polyandra*, the taxon recorded as *B. polyandra* near Visakhapatnam from Andhra Pradesh by Dutt<sup>3</sup> and the one created as a new species of *Acroblastum*, *A. ambavanense*, by Venkatareddi<sup>4</sup> from Poona District are essentially in agreement with the description of *Balanophora abbreviata* Blume (see Bertel Hansen<sup>5</sup>, 1972). *B. polyandra* Griff. is distinctly dioecious with actinomorphic male flowers and tri-porate pollen as against the monoecious *B. abbreviata* Blume in which the inflorescence is bisexual, and the male flowers are zygomorphic with polypantoporate pollen.

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#### ON THE PREVALENCE OF ACTINOMYCETES IN THE RHIZOSPHERE OF THE MEDICINAL PLANT, *SOLANUM KHASIANUM*

ACTINOMYCETES have received little attention in relation to the rhizosphere phenomenon in crop plants<sup>1,2</sup> though emphasis has been on their antagonistic potentialities<sup>3-6</sup>. Actinomycetes were suppressed completely in the rhizosphere of medicinal plant, *Rauvolfia serpentina*<sup>7</sup>. In the present report, evidence is presented on the influence of the medicinal plant, *Solanum khasianum* Clarke on the nature of the actinomycetes in the rhizosphere.

The non-rhizosphere (control) and rhizosphere samples of 18 month old *S. khasianum* plants were collected from 0-3, 3-6 and 6-12 inch layers<sup>6</sup> and the actinomycete population was estimated employing Kuster's agar<sup>7</sup>. The morphological grouping of actinomycetes was established following moist

chamber slide culture method<sup>8</sup> and the antagonistic activity of the actinomycetes was assessed by the standard cross streak assay technique employing various bacterial and fungal test organisms.

Actinomycetes were recorded in larger numbers in the rhizosphere of *S. khasianum* and the rhizosphere effect (rhizosphere : soil ratio) varied from 3.9 to 7.1, the maximum being in the top layer (Table I) and these results are similar to earlier

TABLE I

*Actinomycete population in the non-rhizosphere and rhizosphere soils of S. khasianum from different depths*

| Depth<br>(inch) | Population in 10 <sup>4</sup> /g<br>oven dry soil |                        | R : S<br>ratio |
|-----------------|---|------------------------|----------------|
|                 | Rhizosphere<br>(R)                                | Non-rhizosphere<br>(S) |                |
| 0-3             | 43.3  | 6.1                    | 7.1            |
| 3-6             | 23.4  | 5.9                    | 4.0            |
| 6-12            | 21.0  | 5.4                    | 3.9            |

reports on the incidence of actinomycetes in the rhizosphere of perennial plants<sup>2,9</sup> and other crop plants<sup>3-6</sup>. The rhizosphere effect gradually decreased with the increase in depth and this is because of sudden decline in the actinomycete population in the second and third layers which is similar to the results from yellow birch seedlings<sup>9</sup>. The actinomycetes encountered in the rhizosphere soils belonged to genera *Streptomyces* (80.0%) and *Micromonospora* (20.0%) while *Nocardia* was absent whereas in the non-rhizosphere soils all the three genera were recorded. Among the *Streptomyces*, the series *Spira* was dominant followed by series *Rectus flexibilis* in the rhizosphere, while the series *Retinaculum apertum*, *Monoverticillus*, *Monoverticillus-spira* and *Biverticillus* were absent.

Actinomycetes with antibacterial activity alone were more predominant than those with antifungal and combined antibacterial and antifungal activity in both the rhizosphere and control soils (Table II). Similarly the abundance of antagonistic actinomycetes in the rhizosphere of *Citrus* spp. and other plant species<sup>1-5</sup> were reported earlier. Antagonistic actinomycetes were more in the top two layers while they were absent in third layer of the rhizosphere; the reasons for a specific suppression of antagonists in the deeper layer of the rhizosphere are not known. Majority of antagonists from the rhizosphere were specifically inhibitory to *S. aureus* and *P. solanacearum* and *S. cereviceae* whereas those from non-rhizosphere soil were inhibitory to *S. aureus*, *B. subtilis* and *X. malvacearum*.

TABLE II

*The incidence of antagonistic actinomycetes in the non-rhizosphere and rhizosphere soils of S. khasianum at different depths*

(Expressed as % of total actinomycetes population)

| Treatment              | Antagonistic actinomycetes |            |  |
|------------------------|----------------------------|------------|--|
|                        | Antibacterial              | Antifungal | Combined<br>antibacterial<br>and anti-<br>fungal |
| <i>Rhizosphere</i>     |                            |            |  |
| 0-3 inch               | 60.0                       | 20.0       | 20.0   |
| 3-6 inch               | 40.0                       | 20.0       | 20.0   |
| 6-12 inch              | 0                          | 0          | 0  |
| <i>Non-rhizosphere</i> |                            |            |  |
| 0-3 inch               | 45.0                       | 10.0       | 35.0   |
| 3-6 inch               | 45.5                       | 7.5        | 22.0   |
| 6-12 inch              | 50.0                       | 20.0       | 30.0   |

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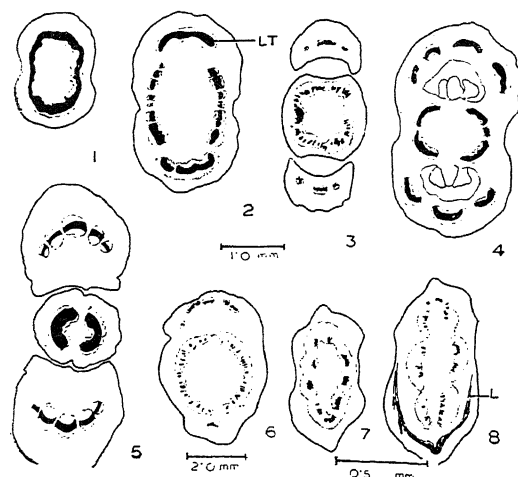
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# PATTERNS OF NODAL ANATOMY IN LOGANIACEAE

ONLY a few<sup>1,2</sup> investigations on the nodal anatomy of family Loganiaceae have appeared so far. The present communication is intended as a supplement to pre-existing literature incorporating the studies from the species belonging to mark 1, 2, 3 and 4 genera. viz., *Strychnos afzelii* Gilg., *S. congolana* Gilg., *S. dinklagei* Gilg., *S. malacoclados* Wright, *S. nux-vomica* L., *S. soubrensis* Hutch. et Dalz., *S. spinosa* Lam., *Nuxia floribunda* Benth., *Gomphostigma virgatum* (L.f.) Baill. and *Buddleia asiatica* Lour.

An internode reveals a complete vascular cylinder (Fig. 1). At the node, a single broad and arc-shaped trace is given off from the vascular cylinder and a gap is formed (Fig. 2). Minor variations are observed during the further course of trace which generally divides into three bundles (Fig. 3), almost of the same size on entering the petiole (e.g., *Strychnos spinosa*). In *S. afzelii* and *S. nux-vomica* the arc-shaped trace first divides itself into three (Fig. 4) middle one being heavier than the two small lateral ones. The latter divides into two each, at the base of the petiole thus supplying 5 bundles to the lamina (Fig. 5). In *S. soubrensis*, however, trace divides into a few, soon after it is given off (Fig. 6).



FIGS. 1-8. Fig. 1. Vascularity of the internode. Fig. 2. Arc-like leaf trace in *S. nux-vomica*. Fig. 3. Leaf trace dividing into 3 in *S. spinosa*. Figs. 4-5. Serial transsections of the node of *S. nux-vomica*. Fig. 6. T.S. node to show leaf trace dividing into many. Figs. 7-8. Serial transsections of the node of *G. virgatum*. (LT—leaf trace, L—lateral).

In *Gomphostigma virgatum* and *Buddleia asiatica* vascular cylinder (Fig. 7) gives off a single trace

which sends one lateral branch each on either side before entering the petiole. The lateral branches divide further to give rise to a branch, each supplying the stipular line before fading away (Fig. 8). In *B. asiatica* laterals are fully consumed.

The data accumulated so far brings forth three patterns of nodal anatomy: (1) multilacunar in *Fagraea* as recorded by Hasselberg<sup>2</sup>, (2) unilacunar single traced with laterals for stipular line, exemplified by *Buddleia asiatica* and (3) unilacunar with single broad, arc-shaped trace, e.g., *Strychnos spinosa*, etc.

The third of these categories needs further consideration since such a broad and arc-shaped trace represents several concrescent strands (Money, Bailey and Swamy, 1950)<sup>3</sup>. Accordingly the following three types indicating probable concrescent nature of arc-shaped trace are evident, (a) single trace dividing into several, (b) single trace dividing into five and (c) single trace dividing into three. The broad, arc-shaped trace, therefore, resulted from the fusion of several strands. Canright<sup>4</sup> considers such a trace as the most evolved among unilacunar types. It is possible that unilacunar condition with a broad arc-like trace represents the ultimate type of nodal pattern in Loganiaceae.

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## ACTIVATED VERMICULITE AS AN INSECTICIDE

EARLIER investigations have indicated that clay minerals could be converted into highly insecticidal substances by activation treatment<sup>1-3</sup>. In the present study, acid and heat activation were carried out on some Indian vermiculites and their insecticidal properties are presented.

Four samples of vermiculite from different states were beneficiated by sedimentation and passed through 100 mesh (Tyler's Standard). Acid activation was carried out by autoclaving the samples with 10 N sulphuric acid at 15 lb/sq. inch pressure having clay acid ratio of 1:6 and washed free of sulphates with deionised water. After drying and pulverizing to 2 $\mu$  fraction, they were heated to 400° C in an electric muffle furnace for 3 hours<sup>1</sup>. The samples were tested for insecticidal activity using *Tribolium castaneum* (Herbst) adults as test insects and the bioassay was conducted in a chamber maintained at 85° F and 56% R.H. Mortality

count was taken after 7 hours exposure and 40 hours post-exposure period. Standard deviation for the replicates was calculated.

The study showed that heat activation alone did not produce any insecticidal activity but on acid heat activation the vermiculites from Karnataka and Bihar developed high insecticidal property (Table I). Vermiculite from Andhra Pradesh also

TABLE I  
Effect of activation on insecticidal activity\*

| Sample No. | Material                               | Unactivated | Heat activated | Acid activated (cold) | †    | Acid heat activated | †    | Acid soluble solids % |
|------------|--|-------------|----------------|-----------------------|------|---------------------|------|-----------------------|
| M24        | Vermiculite (Exfoliated Package waste) | 0           | 3              | 13                    | ±1.2 | 69                  | ±2.6 | 46                    |
| M26        | Vermiculite (Andhra)                   | 0           | 0              | 79                    | ±3.0 | 89                  | ±2.3 | 49                    |
| M27        | Vermiculite (Bihar)                    | 0           | 0              | 64                    | ±2.7 | 92                  | ±3.1 | 51                    |
| M25        | Vermiculite (Karnataka)                | 0           | 0              | 80                    | ±2.3 | 98                  | ±2.4 | 53                    |

\* Per cent insect mortality. † Standard deviation. Degree of freedom 19.

gave good activity whereas pre-exfoliated vermiculite (package waste) on activation treatment showed lower insecticidal activity. It was also observed that samples which had acid solubility of more than 50% showed greater insecticidal property. The acid treatment produced more micro-capillaries on the particle surface and adsorbed lipids from the insect cuticle. The insect mortality was brought about by the resultant desiccation<sup>1,2</sup>.

Acute oral toxicity trials of vermiculite (2–8 g/kg) on albino rats were conducted to determine the mammalian safety. Even high level ingestion did not produce any toxic symptoms in rats for 48 hrs. The study also indicated that vermiculite is a safe and promising mineral for controlling stored-product insects.

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### INFLUENCE OF ALDRIN ON RADIO-CARBON (<sup>14</sup>C-GLUCOSE) INCORPORATION BY TWO RHIZOBIUM SPECIES

APPLICATION of various insecticides to soil to control soil-borne insect pests is a common practice in modern agriculture. Some of these insecticides, especially chlorinated hydrocarbons, persist in soil for a long time. The effect of such persistent type of insecticides on the beneficial microorganisms in soil, such as *Rhizobium*, a symbiotic nitrogen fixing group of bacteria, is little understood. Oblisami *et al.*<sup>4</sup> reported that the insecticides endrin, carbofuran and disulfoton inhibited to varying degrees, the growth *in vitro* of *Rhizobium* from red gram; however, Balasubramanian<sup>1</sup> observed that lindane, upto 5 ppm concentration, did not affect the growth of *Rhizobium japonicum in vitro* but altered the carbon (glucose) metabolism of the organism. The effect of Aldrin (1, 2, 3, 4, 10, 10-hexachloro 1, 4, 4a, 5, 8, 8a-hexahydro-endo 1, 4-exo-5, 8-dimethanonaphthalene), a common soil applied insecticide, on the radiocarbon (<sup>14</sup>C-glucose) assimilation *in vitro* by two species of *Rhizobium* is reported in this communication.

*Rhizobium trifolii* from lucerne (*Medicago sativa* L.) and *Rhizobium* sp. from black gram (*Phaseolus mungo* var. *radiatus* Linn.) were grown in malt extract medium<sup>2</sup> for 48 hr. One ml of this culture was added to 99 ml of the medium contained in 250 ml Erlenmeyer flasks. Calculated quantities of Aldrin (prepared from 'Aldrex-20 EC' formulated by Shell chemicals) were added to each flask so as to obtain 1 ppm (normal level) and 2 ppm (two-fold level) final concentrations of the active ingredient. Duplicates were maintained under each treatment with appropriate controls. The flasks were then incubated on a gyratory shaker at 25°C. After 48 hr incubation, to each flask was added 1 ml of an aqueous solution of radio-active (<sup>14</sup>C) glucose (uniformly labelled) of specific activity 0.05 mc/ml (22 mc/mM of glucose) using an automatic micro-syringe and again incubated for 3 hr. The cells were then harvested by centrifuging at 27,000 g at 5°C for 10 min. with repeated washings with physiological saline.

One part of the cell pellet was taken in an aluminium planchet, demoi-stened under infra-red heat and the radioactivity monitored with a gas flow proportional counting system. Of the remaining portion of the cell mass, equal quantities (on wet weight basis) of cells of the two *Rhizobium* species were extracted with various solvents as described by Kamen<sup>3</sup> and the following cellular fractions were obtained in succession: (1) cold-TCA soluble fraction, (2) alcohol soluble fraction, (3) alcohol-ether soluble fraction, (4) hot-TCA

TABLE I

Influence of Aldrin on the radio-carbon ( $^{14}\text{C}$ -glucose) incorporation by two species of *Rhizobium*

| Treatment                            | Total activity<br>(whole cells)<br>cpm/mg dry<br>cells | % radioactivity incorporated in |                                |                                      |                                |                      |
|--------------------------------------|--|---------------------------------|--------------------------------|--------------------------------------|--------------------------------|----------------------|
|                                      |  | Cold TCA<br>soluble<br>fraction | Alcohol<br>soluble<br>fraction | Alcohol-ether<br>soluble<br>fraction | hot-TCA<br>soluble<br>fraction | Insoluble<br>residue |
| <i>Rhizobium trifolii</i> (Lucerne): |  |                                 |                                |                                      |                                |                      |
| No insecticide (control)             | 39.25±1.91   | 27.39                           | 20.09                          | 11.52                                | 37.23                          | 3.77                 |
| Aldrin 1 ppm                         | 56.11±2.52   | 25.24                           | 20.78                          | 12.00                                | 37.11                          | 4.87                 |
| „ 2 ppm                              | 52.90±0.95   | 33.45                           | 15.55                          | 6.67                                 | 37.20                          | 7.12                 |
| <i>Rhizobium</i> sp. (Blackgram):    |  |                                 |                                |                                      |                                |                      |
| No insecticide (control)             | 20.41±0.39   | 37.21                           | 18.13                          | 17.08                                | 18.72                          | 8.86                 |
| Aldrin 1 ppm                         | 26.62±0.71   | 46.40                           | 15.66                          | 8.95                                 | 17.20                          | 11.79                |
| „ 2 ppm                              | 26.54±0.63   | 48.28                           | 17.19                          | 6.89                                 | 19.05                          | 8.60                 |

cpm: Counts per minute.

soluble fraction and (5) insoluble residue. One ml of each fraction was transferred to an aluminium planchet, evaporated to dryness under infra-red heat and the radioactivity monitored.

The per cent distribution of radioactivity in the cellular fractions of the two rhizobia is presented in Table I. The total radioactivity incorporated into the whole cells increased significantly with the insecticide treatment in both the rhizobial species indicating a general increase in the carbon ( $^{14}\text{C}$ -glucose) assimilation by the organism.

It is clear from the results that Aldrin treatment altered the  $^{14}\text{C}$ -glucose assimilation in different fractions of the cellular constituents of the two species of *Rhizobium* and the effect varied with the level of the insecticide. Balasubramanian<sup>1</sup> observed in *R. japonicum* that lindane, a gamma isomer of benzene hexachloride (BHC), altered the incorporation of radio-carbon ( $^{14}\text{C}$ -glucose) into the different constituents of the growing cells. The observed increase in the percentage incorporation of radio-carbon induced by the insecticide treatment, in the cold-TCA soluble fraction and the insoluble residue, presumably composed of polysaccharides and protein respectively, indicate increased synthesis of carbohydrates and proteins in the cells, at the cost of lipid synthesis (alcohol-ether soluble fraction), with no appreciable change in other cellular constituents, viz., alcohol soluble fraction (cell wall and cytoplasmic membranes) and hot-TCA soluble fraction (nucleic acids), in the two *Rhizobium* species. The exact mode of action of Aldrin on the carbon metabolism of *Rhizobium* spp. and the effect of such carbon assimilation process, as affected by the insecticide on the *Rhizobium*-legume symbiosis, are yet to be worked out.

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#### EFFECT OF DIFFERENT CONCENTRATION OF ETHREL (2-CHLOROETHYL PHOSPHONIC ACID) ON THE PROPERTIES OF BANANA FRUITS DURING THE ARTIFICIAL RIPENING

THE plant growth activity of ethrel has been attributed primarily to its ability to release ethylene to plant tissues<sup>15-16</sup>. Burg<sup>3</sup> and others<sup>8-10</sup> have demonstrated the dramatic influence of ethylene on fruit ripening and maturity. However, a simple dipping procedure with ethrel would be of interest in many parts of the world. Postharvest dipping for 30 seconds to 3 minutes or spraying the fruit with ethrel has promoted ripening of bananas at 500 to 2,000 ppm<sup>5-14</sup>, pears at 250 to 1,000 ppm<sup>3-6, 11</sup>, pecans at 6,000 ppm<sup>9</sup> and mangoes at 100 to 10,000 ppm<sup>4</sup>. The present investigation is aimed at studying the effect of different concentrations of ethrel on fruit quality of "Maghraby" banana, during the artificial ripening.

*Materials and Methods.*—Eight mature bunches of "Maghraby" banana fruits (*Musa sapientum* var. Gros Michel), were used in this investigation,

representing four treatments of two bunches each. The banana bunches were sprayed with aqueous solutions of ethrel, at 0, 500, 1,000 or 2,000 ppm and were kept at room temperature (70–75° F). Determination of some physical and chemical characteristics of the fruits such as colour development, peelability, total soluble solids, total sugars, reducing sugars, non-reducing sugars were carried out at three day intervals. Extraction and determination of sugars were carried out according to the modified Schaffer-Hartmann<sup>13</sup> methods as described by Said<sup>12</sup>.

#### RESULTS AND DISCUSSION

1. *Colour Development.*—The colour development was estimated according to Fruit Dispatch Company Colour Chart<sup>7</sup>. The rate of colour development (Table I) was more rapid in fruits treated with 2,000 and 1,000 ppm than those treated with 500 and 0 ppm ethrel. These results are in agreement with those reported by Abou Aziz and Abd-El-Wahab<sup>1</sup>.

TABLE I  
*Effect of different concentrations of ethrel on colour development during the artificial ripening of banana fruits at 70–75° F*

| Period of ripening in days | Grade No. after treatment |         |           |           |
|----------------------------|---------------------------|---------|-----------|-----------|
|                            | Control                   | 500 ppm | 1,000 ppm | 2,000 ppm |
| 0                          | 1                         | 1       | 1         | 1         |
| 3                          | 1                         | 3       | 5         | 5         |
| 6                          | 1                         | 5       | 6         | 6         |
| 9                          | 2                         | 6       | 7         | 7         |
| 12                         | 3                         | 7       | 8         | 8         |
| 15                         | 4                         | ..      | ..        | ..        |
| 18                         | 5                         | ..      | ..        | ..        |

- |                            |                                   |
|----------------------------|-----------------------------------|
| 1. Green.                  | 5. Green tip.                     |
| 2. Green, trace of yellow. | 6. All yellow.                    |
| 3. More green than yellow. | 7. Yellow-flecked with brown.     |
| 4. More yellow than green. | 8. Yellow with large brown areas. |

TABLE II

*Effect of different concentrations of ethrel on the peeling condition, during the artificial ripening of banana fruits*

| Period of ripening in days | Treatments   |              |              |              |
|----------------------------|--------------|--------------|--------------|--------------|
|                            | Control      | 500 ppm      | 1,000 ppm    | 2,000 ppm    |
| 0                          | Unpeeling    | Unpeeling    | Unpeeling    | Unpeeling    |
| 3                          | do.          | Hard peeling | Peeling      | Peeling      |
| 6                          | do.          | Peeling      | Easy peeling | Easy peeling |
| 9                          | Hard peeling | Easy peeling | do.          | do.          |
| 12                         | do.          | do.          | do.          | do.          |
| 15                         | Peeling      | ..           | ..           | ..           |
| 18                         | do.          | ..           | ..           | ..           |

2. *Peeling Condition.*—It seemed from Table II, that fruits were still hard to peel till the first sign of ripening was noticed. The time required to peel the banana fruits easily was shortened by 3–6 days depending on the concentration of ethrel. It is interesting to note that the colour test and peeling condition form a good criterion for evaluating the ripening of banana fruits.

3. *Total Soluble Solids.*—Data in Table III show a progressive and consistent increase in the total soluble solids during the ripening in the treated fruits. The increase of total soluble solids during ripening is due to the starch hydrolysis<sup>1,2</sup>.

4. *Total Sugars.*—The effect of different concentrations of ethrel on total sugars content of banana fruits is shown in Table IV. Total sugars reflect a gradual increase during the ripening of the banana fruits until the end of ripening stage. This was true in both treated and untreated fruits.

TABLE III

*Effect of different concentrations of Ethrel on the total soluble solids during the artificial ripening of banana fruits*

| Period of ripening (in days) | % total soluble solids |         |           |           |
|------------------------------|------------------------|---------|-----------|-----------|
|                              | Control                | 500 ppm | 1,000 ppm | 2,000 ppm |
| 0                            | 3.6                    | 3.6     | 3.6       | 3.6       |
| 3                            | 3.7                    | 5.6     | 7.7       | 9.0       |
| 6                            | 4.0                    | 8.2     | 15.0      | 16.4      |
| 9                            | 5.3                    | 15.2    | 16.7      | 18.8      |
| 12                           | 8.3                    | 16.9    | 18.0      | 20.9      |
| 15                           | 11.1                   | ..      | ..        | ..        |
| 18                           | 11.5                   | ..      | ..        | ..        |

The rate of increase in the total sugars was faster in the treated fruits than in the untreated.

TABLE IV

Effect of different concentrations of ethrel on total sugars (reducing and non-reducing) during the artificial ripening of banana fruits

| Period of ripening (in days) | Control |      |         | 500 ppm |       |         | 1,000 ppm |       |         | 2,000 ppm |       |         |
|------------------------------|---------|------|---------|---------|-------|---------|-----------|-------|---------|-----------|-------|---------|
|                              | T.S     | R.S  | Non-R.S | T.S     | R.S   | Non-R.S | T.S       | R.S   | Non-R.S | T.S       | R.S   | Non-R.S |
| 0                            | 2.3     | 0.9  | 1.4     | 2.3     | 0.9   | 1.4     | 2.3       | 0.9   | 1.4     | 2.3       | 0.9   | 1.4     |
| 3                            | 2.6     | 1.1  | 1.5     | 5.31    | 3.01  | 2.3     | 6.13      | 4.1   | 2.03    | 8.73      | 6.5   | 2.23    |
| 6                            | 3.7     | 1.71 | 1.99    | 7.82    | 4.52  | 3.3     | 14.04     | 10.14 | 3.9     | 14.53     | 10.66 | 3.87    |
| 9                            | 5.81    | 2.8  | 3.01    | 14.6    | 10.73 | 3.87    | 16.88     | 12.3  | 4.58    | 18.03     | 13.26 | 4.77    |
| 12                           | 8.11    | 4.21 | 3.9     | 15.9    | 11.33 | 4.57    | 16.81     | 11.83 | 4.98    | 19.30     | 14.21 | 5.09    |
| 15                           | 10.8    | 7.0  | 3.8     | ..      | ..    | ..      | ..        | ..    | ..      | ..        | ..    | ..      |
| 18                           | 11.9    | 8.1  | 3.8     | ..      | ..    | ..      | ..        | ..    | ..      | ..        | ..    | ..      |

T.S: total sugars,

R.S: reducing sugars,

Non-R.S: non-reducing sugars.

Maximum value of total sugars was high in the fruits treated with 2,000 ppm than those treated with 1,000 or 500 ppm of ethrel. It was also noticed that the reducing and the non-reducing sugars take a similar trend. Reducing sugars represent the major portion of the total sugars during the course of artificial ripening. This may be attributed to the early ripening process in the treated fruits. Fruits treated with 2,000 ppm of ethrel reflect a higher value of reducing and non-reducing sugars than those treated with 500 and 1,000 ppm.

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## SHORT SCIENTIFIC NOTES

### Fertility in Relation to Different Haemoglobin Variants in Hariana Cows

Several attempts have been made to correlate haemoglobin types with fertility in sheep<sup>1,2</sup>. However, there appears no published information on the possible association between Hb types and fertility in Zebu cows. The present note reports the same. 645 Hariana cows of different Hb phenotypes were randomly bred to different bulls irrespective of their Hb types over a period of 4 years. Hb AA cows produced significantly more calves (62.6%) than either Hb AB (47.5%) or Hb BB (51.1%) animals. In addition, Hb AA animals appeared to calve at a significantly earlier age ( $1237 \pm 28$  days) than the Hb AB ( $1336 \pm 32$  days) and Hb BB ( $1340 \pm 25$  days) types. Calving interval was also slightly, though not significantly, shorter in Hb AA cows. Since Hb type in cattle is inherited as a simple Mendelian trait<sup>3</sup>, advantage can perhaps be taken of this apparent association between Hb types and reproductive efficiency for improving fertility in Zebu cattle through controlled breeding based on this physiological trait.

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### Fertility in Relation to Red Cell Potassium Types in Murrah Buffaloes

A recent study<sup>1</sup> has indicated that the distribution of red cell potassium in buffaloes is genetically controlled. The possible association between red cell K<sup>+</sup> types and reproductive performance in Murrah buffaloes is reported here. The study was carried out with 256 buffalo cows and 11 buffalo bulls over a period of 3 years. Female fertility was nearly identical in HK (48.4%) and LK type (45.7%) of buffalo cows. In contrast, male fertility appeared to be related to red cell K<sup>+</sup> types, the trend being in favour of LK animals. Gross initial motility (LK — 2.02 and H.K. — 2.6), live sperm concentration  $\times 10^6/\text{ml}$  (LK — 1348, HK — 979) and fructolytic index (HK — 1.75; LK — 1.40) were significantly more in LK type than in HK type of bulls. Moreover, the LK bulls

appeared to be significantly more fertile (50.4%) than the HK bulls (33.8%). The no. of services per conception was not significantly different in the two groups (HK 1.86; LK 1.75). The present findings point to the practical possibility of selection of potentially more fertile young bulls at a very early age on the basis of red cell K<sup>+</sup> type.

Dept. of Physiology,

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U.P. College of Veterinary

Science,

Mathura (India), July 4, 1974.

1. Sengupta, B. P., *J. agric. Sci.*, 1974, 82, 559.

### Additions to the Host Range of Bottle Gourd Mosaic Virus

A mosaic disease of bottle gourd (*Lagenaria leucantha*) is wide spread in Aligarh. Detailed studies with regard to the host range, symptomatology, thermal inactivation, longevity *in vitro*, dilution end point, transmission behaviour revealed that the virus is identical to the one described by Vasudeva *et al.*<sup>2</sup> and Shanker *et al.*<sup>1</sup>. In the host range studies, however, *Commelina nudiflora*, *Mukia maderaspatana* and *Salvia* sp. proved to be additional hosts of the virus. All these plants showed visible symptoms and the virus was recovered on back inoculation to *L. leucantha*. Though no visible symptoms were evoked on *Cucurbita pepo*, *Gomphrena globosa* and *Impatiens balsamina* yet on back inoculation virus was recovered and thus they served as symptomless carrier of the virus. *Datura stramonium* was described earlier (Vasudeva *et al.*<sup>2</sup>) as symptomless carrier, however, it was proved to be non-host in the present studies.

Department of Botany,

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Aligarh 202001, India,

September 5, 1974.

1. Shankar, G., Nariani, T. K. and Prakash, N., *Indian J. Microbiol.*, 1971, 11, 43.
2. Vasudeva, R. S., Raychaudhuri, S. P. and Singh, J., *Indian Phytopath.*, 1949, 2, 180.

### Record of the Greenhouse Whitefly *Trialeurodes vaporariorum* (Westwood) on Tomato

The greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) was first reported to occur in India by David<sup>1</sup> infesting potato (*Solanum tuberosum*) at Thummanatty (Nilgiris). Recently, the

insect was noticed for the first time to infest severely tomato (*Lycopersicon esculentum*) plants and to a mild extent French bean (*Glycine max*) at Kotagiri (Nilgiris).

The species being a plastic one exhibits conspicuous variation which can be correlated with the physical properties of the leaves it inhabits. The specimens collected agreed with the structural features of the pupal cases that occur on moderately hairy leaf, except for the difference in the length of the 8th abdominal setae which were about  $87\mu$  long as against 10 to  $20\mu$  in the normal form.

The occurrence of the whitefly at Thummanatty and Kotagiri indicates that the species is widely prevalent in the Nilgiris and a thorough search may add to the discovery of additional hosts.

The authors extend their sincere thanks to Prof. T. R. Subramanian, for the facilities provided. Dept. of Entomology, A. V. NAVARAJAN PAUL.  
Tamil Nadu Agril. Univ., B. VASANTHARAJ DAVID.  
Coimbatore 641003, September 5, 1974.

1. David, B. V., *Studies on South Indian Aleyrodidae*, Doctoral Thesis, Tamil Nadu Agricultural University, Coimbatore, 1971.

#### Grafting Behaviour Between *Lycopersicon esculentum* L. Var. Marglobe and *Solanum* spp. (*Solanum torvum* and *S. melongena* L.)

Grafting has been an important method of overcoming incompatibility barriers in crosses between species of the genus and genera of the family. Hely *et al.* (1953)<sup>1</sup>, grafted *Trifolium repens* on to *T. ambiguum* and achieved good result. Later, Nirk (1959)<sup>2</sup> obtained eight self sterile interspecific hybrids by grafting prior to crossing, *L. esculentum* and *L. peruvianum*. In the present study, wedge grafting was used to make grafts between *Solanum* spp. and *L. esculentum*. *Solanum torvum* which is a perennial species with an elaborate root system and which is a hardy crop, was used as stock and *L. esculentum* was used as scion. The objects were to examine the possibilities of making *L. esculentum* perennial, increase its life span, make it drought and disease (wilt) resistant. Observations revealed that stock-scion establishment was early, remained for a longer period with profuse flowering in *S. melongena* and *L. esculentum* grafts compared to *S. torvum* and *L. esculentum*. In addition, the number of successful grafts were more in the former grafting than in the latter. But in both the cases the grafts could not be made to remain

perennial. Pollen studies in the grafted plants indicated no significant pollen sterility. A plausible explanation for this failure may be the inability of *L. esculentum* to maintain the seasonal hormonal balance like *S. torvum* which is perennial.

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Hebbal, Bangalore 560024. G. SHIVASHANKAR.  
November 16, 1974.

1. Hely, F. W., Bonnier, CH. and Manil, P., *Nature*, 1953, 171, 884.
2. Nirk, H., *Ibid.*, 1959, 184, 1819.

#### ANNOUNCEMENTS

Nominations for Science Academy Medals for young Scientists for 1975 are invited. The last date is February 28, 1975. For details please apply to the Executive Secretary, Indian National Science Academy, New Delhi-1.

#### Award of Research Degree

Karnatak University, Dharwar, has awarded the Ph.D. degree in Chemistry to Mrs. Renuka Rani Nagendrappa for her thesis entitled "Some Studies of Donor Acceptor Complexes"; Ph.D. degree in Zoology to (Miss) K. Dakshayani for her thesis entitled "Influence of Nutrition, Temperature, Groupsize and Photoperiod on Nymphal Growth and Development of the Cricket, *Pleaeiogryllus guttiventris* Walker"; Ph.D. degree in Chemistry to Shri Mohan Ramanath Shanbhag for his thesis entitled "Studies in Fatty Acids"; Ph.D. degree in Zoology to Shri Srinivas Kishanrao, Saidapur, for his thesis entitled "Studies on the Gonads of *Rana gyanophlyctis*, *Rana tigrina* and *Bufo melanostictus* (Amphibia) with reference to steroidogenic cellular sites"; Ph.D. degree in Geology to Shri Veerappa Chanbasappa Chavadi for his thesis entitled "Geology of the mafic and the other associated rocks of Savantavadi area, Ratnagiri District, Maharashtra State".

Osmania University, Hyderabad, has awarded the Ph.D. degree in Physics to Shri Sarada Prasad Mohanty for his thesis entitled "Theory of Orbital Susceptibility of Metals with complicated crystal structures"; Ph.D. degree in Physics to Shri S. P. Mallikarjun Rao for his thesis entitled "Ultrasonic Studies in Liquids by Reverberation Technique"; Ph.D. degree in Chemistry to Shri P. K. Sai Prakash for his thesis entitled "A Kinetic Study of some Aspects of Oxidation of Organic Substrates".

## INFORMATION TO CONTRIBUTORS

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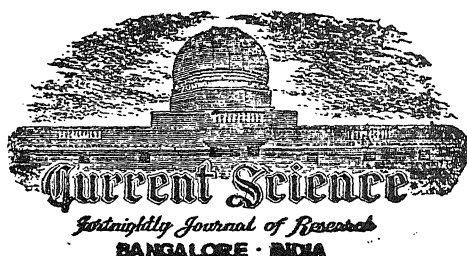
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## ABSTRACT

A normal coordinate analysis of sulphamide  $\text{SO}_2(\text{NH}_2)_2$  molecule is carried out using the 'characteristic set of valence coordinates' introduced by Herranz and Castano<sup>1</sup> and a set of potential energy constants is reported. Mean vibrational amplitudes, coriolis constants and thermodynamic quantities for a harmonic oscillator approximation, at different temperatures are also evaluated.

## INTRODUCTION

THE study of sulphamide is of general interest because of its marked acid properties and its ability to form salts of the type  $\text{SO}_2(\text{NHAg})_2$ . The exact nature of the force field of a molecule cannot be determined unambiguously from the vibrational frequencies alone. To fix the force fields completely, additional data like mean vibrational amplitudes, coriolis constants and thermodynamic properties are often necessary. However, approximate force fields can be determined using the kinematic method suggested by Herranz and Castano<sup>1</sup>. The present paper deals with the analysis of molecular force fields of the sulphamide molecule using the above method.

## 1. POTENTIAL ENERGY CONSTANTS

The molecule sulphamide belongs to  $C_{2v}$  point group nearly in agreement with X-ray results<sup>2</sup> and with the fundamental vibrational frequencies<sup>2</sup> falling under the irreducible representation  $\tau = 4a_1 + 1a_2 + 2b_1 + 2b_2$ . From the vibrational frequencies<sup>2</sup> and structural parameters<sup>3</sup> of this molecule, the symmetry coordinates and the corresponding inverse kinetic energy G matrix elements were constructed. The potential energy F matrix elements were obtained by Herranz and Castano method<sup>1</sup>.

$$L^{-1} = BM^{\dagger}B^*$$

where B is the orthogonal matrix which diagonalises G. M is a diagonal matrix whose elements are the reciprocals of the eigenvalues of G. The asterisk refers to the transpose. The symmetry force constant matrix is given by

$$F = L^{-1*} \Omega L^{-1}$$

where  $\Omega$  is a diagonal matrix with

$$\Omega_k = 4\pi^2 c^2 \gamma_k^2$$

$\gamma_k$  is the  $k$ th frequency ( $\text{cm}^{-1}$ ) and  $c$  is the velocity of light ( $\text{cm/sec}$ ). The symmetry coordinates in the present work are essentially the same as those of Ramaswamy and Jayaraman<sup>4</sup> where the constants  $a$ ,  $b$ ,  $c$  and  $d$  have the

following values :  $a = 0.852099$ ,  $b = 0.977023$ ,  $c = 0.420161$ , and  $d = 1$ .

Also  $D = S - O$ ,  $d = S - (\text{NH}_2)$ ,  $a = \text{O}\hat{S}\text{O}$   
 $\beta = (\text{NH}_2) - \hat{S} - (\text{NH}_2)$  and  $\gamma = (\text{NH}_2) - \hat{S} - O$ .

The valence force constants thus evaluated are presented in Table I.

TABLE I

Valence force constants of sulphamide molecule

| Bond stretching and bond-bond interactions* | Bond-angle interactions** | Angle-angle interactions† |
|---|---------------------------|---------------------------|
| $f_D$ 12.1114‡                              | $f_{DZ}$ 1.8276           | $f_a$ 1.3747              |
| $f_d$ 6.3103                                | $f_{d\beta}$ 1.1146       | $f_\beta$ 1.9960          |
| $f_{DD}$ -0.3556                            | $f_{D\gamma}$ -2.4984     | $f_\gamma$ 1.5920         |
| $f_{dd}$ 0.4780                             | $f_{d\gamma}$ 1.3237      |                           |
| $f_{d\alpha}$ 0.5434                        |                           |                           |

\* in m.dynes  $\text{\AA}^{-1}$  ; \*\* in m.dynes  $\text{rad}^{-1}$  ;

† in m.dynes  $\text{\AA}^{-1} \text{rad}^{-2}$  ;

‡ This number of significant figures is retained for internal consistency in the calculations.

## 2. MEAN AMPLITUDE OF VIBRATIONS

The important vibrational mean amplitudes of different bonds were calculated by the method of Cyvin<sup>5</sup> and the nonbonded ones by the method of Ramaswamy *et al.*<sup>6</sup> and are presented in Table II.

TABLE II

 Mean vibrational amplitudes of sulphamide  $\text{SO}_2(\text{NH}_2)_2$  molecule of  $\text{XO}_2\text{Y}_2$  type

|        |                      |
|--------|----------------------|
| X = O  | 0.03690 $\text{\AA}$ |
| X - Y  | 0.04400              |
| Y .. Y | 0.05936              |
| O .. Y | 0.06488              |
| O .. O | 0.05548              |

(Dotted line represents nonbonded distance)

## 3. CORIOLIS COUPLING CONSTANTS

The coriolis coupling constants ( $\xi$ ) were evaluated using the relation given by Meal and Polo<sup>7</sup> and are presented in Table III.

TABLE III  
Coriolis coupling coefficients of sulphamide

| coupling species | $\zeta_{ij}^x$ | coupling species | $\zeta_{ij}^y$ | coupling species | $\zeta_{ij}^z$ |
|------------------|----------------|------------------|----------------|------------------|----------------|
| $a_1 \times b_2$ | $\zeta_{18}^x$ | $a_1 \times b_1$ | $\zeta_{16}^y$ | $a_1 \times a_2$ | $\zeta_{15}^z$ |
|                  | 0.0055         |                  | 0.0182         |                  | 0.6408         |
|                  | $\zeta_{26}^x$ |                  | $\zeta_{26}^y$ |                  | $\zeta_{15}^z$ |
|                  | 0.0077         |                  | 0.0196         |                  | -0.5546        |
|                  | $\zeta_{38}^x$ |                  | $\zeta_{36}^y$ |                  | $\zeta_{25}^z$ |
|                  | 0.7421         |                  | 0.6599         |                  | 0.5309         |
|                  | $\zeta_{48}^x$ |                  | $\zeta_{46}^y$ |                  | $\zeta_{45}^z$ |
|                  | -0.6371        |                  | 0.7559         |                  | 0.0785         |
|                  | $\zeta_{19}^x$ |                  | $\zeta_{17}^y$ | $b_2 \times b_2$ | $\zeta_{65}^z$ |
| $a_2 \times b_1$ | 0.4618         | $a_2 \times b_2$ | 0.6920         |                  | -0.0023        |
|                  | $\zeta_{29}^x$ |                  | $\zeta_{27}^y$ |                  | $\zeta_{69}^z$ |
|                  | -0.4408        |                  | -0.4744        |                  | 0.8000         |
|                  | $\zeta_{39}^x$ |                  | $\zeta_{37}^y$ |                  | $\zeta_{78}^z$ |
|                  | -0.4914        |                  | -0.4711        |                  | 0.6956         |
|                  | $\zeta_{49}^x$ |                  | $\zeta_{47}^y$ |                  | $\zeta_{79}^z$ |
|                  | -0.5982        |                  | 0.5682         |                  | -0.4362        |
|                  | $\zeta_{56}^x$ |                  | $\zeta_{58}^y$ |                  |                |
|                  | -0.1929        |                  | 0.1803         |                  |                |
|                  | $\zeta_{57}^x$ |                  | $\zeta_{59}^y$ |                  |                |
|                  | -0.2412        |                  | 0.1252         |                  |                |

TABLE IV  
Thermodynamic properties of sulphamide molecule

| T° K    | $(H^\circ - E_0)/T^*$ | $-(F^\circ - E_0)/T^*$ | $S^\circ$ | $C_p^\circ$ |
|---------|-----------------------|------------------------|-----------|-------------|
| 100°    | 8.0398                | 48.1875                | 56.2273   | 8.4992      |
| 200°    | 9.1192                | 54.0297                | 63.1469   | 12.1276     |
| 298.16° | 10.7032               | 57.9618                | 68.6650   | 15.6004     |
| 300°    | 10.7334               | 58.0277                | 68.7611   | 15.6571     |
| 400°    | 12.3106               | 61.3351                | 73.6457   | 18.2827     |
| 500°    | 13.7024               | 64.2354                | 77.9378   | 20.1477     |
| 600°    | 14.8925               | 66.8418                | 81.7343   | 21.4626     |
| 700°    | 15.9017               | 69.2154                | 85.1171   | 22.3999     |
| 800°    | 16.7587               | 71.3963                | 88.1549   | 23.0807     |
| 900°    | 17.4905               | 73.4135                | 90.9040   | 23.5857     |
| 1000°   | 18.1200               | 75.2897                | 93.4097   | 23.9681     |

$H^\circ$  = heat content,  $F^\circ$  = free energy,  $S^\circ$  = Entropy,  $C_p^\circ$  = heat capacity,  $E_0$  = Energy per gm. mol of the perfect gas at  $T = 0^\circ$  Cal deg<sup>-1</sup> mol<sup>-1</sup>.

#### 4. THERMODYNAMIC PROPERTIES OF SULPHAMIDE

A set of thermodynamic quantities is also calculated and presented in Table IV.

Principal moments of inertia calculated are

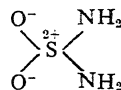
$$I_{xx} = 97.4299 \text{ a.m.u.}\text{\AA}^2, \quad I_{yy} = 87.0582 \text{ a.m.u.}\text{\AA}^2, \\ I_{zz} = 102.2358 \text{ a.m.u.}\text{\AA}^2.$$

#### RESULTS AND DISCUSSION

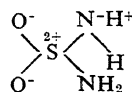
In these calculations, the approximation that the  $(\text{NH}_2)$  group is a point mass, has been made. The mean amplitude value for nonbonded distance  $\text{O} \cdots \text{O}$  (0.05548 Å) agrees favourably with others<sup>8,9</sup> for similar types of molecules. The reliability of the  $\zeta$  (zeta) of Table III is confirmed since they obey the following sum rules.

$$(\zeta_{18}^x)^2 + (\zeta_{28}^x)^2 + (\zeta_{38}^x)^2 + (\zeta_{48}^x)^2 = 1 \\ (\zeta_{19}^x)^2 + (\zeta_{29}^x)^2 + (\zeta_{39}^x)^2 + (\zeta_{49}^x)^2 = 1 \\ (\zeta_{16}^y)^2 + (\zeta_{26}^y)^2 + (\zeta_{36}^y)^2 + (\zeta_{46}^y)^2 = 1 \\ (\zeta_{15}^z)^2 + (\zeta_{25}^z)^2 + (\zeta_{35}^z)^2 + (\zeta_{45}^z)^2 = 1$$

At present no experimental data are available to check the calculations of coriolis constants, mean amplitudes and thermodynamic properties reported in this paper. From the bond order calculation<sup>10</sup> [S-O (2.2), S-N (1.7)] supporting the resonance structure, the valence bond structure may be written



In each  $>\text{N-H}$  bond there may be resonance between the covalent and the ionic  $>\text{N}^+\text{H}^-$  states and the formation of negative charge on the nitrogen atom increases the stability of the molecule in view of the gain of electrostatic energy in the S-N bond. Thus the contribution of the structure



may be large in view of the increased stability produced by the alternative positive and negative charges. The marked acid properties of sulphamide and its ability to form salts of the type  $\text{SO}_2(\text{NHAg})_2$  may be in agreement with this structure.

The authors express their thanks to Dr. K. N. Kuchela and to Prof. M. A. Venkatachar for their interest in this work.

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## NEUTRAL COMPONENTS OF *THESPEsia POPULNEA* FLOWERS

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### ABSTRACT

The flowers of *Thespesia populnea* have been found to contain nonacosane, lupenone, myricyl alcohol, lupeol,  $\beta$ -sitosterol and  $\beta$ -sitosterol- $\beta$ -D-glucoside.

THE flowers of *Thespesia populnea* have been investigated a number of times in the past, with reference to the polyphenolic components present<sup>1-3</sup>. When the material is extracted in succession with petroleum ether (60–80°), acetone and alcohol, the flavonoids as aglycones and glycosides are found largely in the alcoholic extract<sup>4</sup>. The petroleum ether extract has yielded (+) gossypol as the main component<sup>5</sup>. The present note gives particulars of neutral components now isolated.

The flowers of *Thespesia populnea* (3 kg) were exhaustively extracted with petroleum ether (60–80°) and the extract deposited the yellow pigment (+) gossypol, reported earlier by Datta *et al.*<sup>5</sup>. The petroleum ether was completely removed and the residue taken up in ether. It was extracted with sodium hydroxide. Acidification of the alkaline extract gave a little more of (+) gossypol. On evaporation of the remaining ether extract a dark coloured semi-solid was obtained. It was adsorbed on silica gel and chromatographed on a column of the same whereby the following compounds were obtained.

Compound A, eluted with petroleum ether, had m.p. 64–65° (300 mg, from acetone). Its I.R. spectrum showed that it was aliphatic in nature. It did not answer Liebermann-Burchard test and moved to solvent front on TLC in *n*-hexane. Its mass-spectrum showed a very weak parent peak at *m/e* 408 and showed a cluster of peaks 14 units ( $-\text{CH}_2$ ) apart. The largest peak in each cluster represented  $\text{C}_n\text{H}_{2n+1}$  fragment which was accompanied by  $\text{C}_n\text{H}_{2n}$  and  $\text{C}_n\text{H}_{2n-1}$  peaks. Very

intense peaks were for C-4 and C-5 units and the fragment intensity decreased in a smooth curve up to  $(\text{M}^+-\text{C}_2\text{H}_5)$ . The  $(\text{M}^+-\text{CH}_3)$  peak was very small. The compound was identified as nonacosane.

Compound B, eluted with petroleum ether-benzene (1 : 1), had m.p. 168–170° (from acetone, 40 mg),  $[\alpha]_D - 40^\circ$ ;  $\nu_{\max}$  1725  $\text{cm}^{-1}$  (C=O) and gave a phenyl hydrazone, m.p. above 300°. Liebermann-Burchard test was positive. The compound was identified as lupenone.

Compound C, eluted with benzene, had m.p. 80° (from acetone, 200 mg),  $\nu_{\max}$  3500  $\text{cm}^{-1}$  (OH) and gave an acetate, m.p. 67–69°. The mass-spectrum of the acetate showed the parent peak at *m/e* 480 and the base peak at 421, confirming the presence of the acetate group ( $\text{M}^+-59$ ). It also showed a cluster of peaks 14 units ( $-\text{CH}_2$ ) apart. Very intense peaks were for C-6, C-7 and C-8 units. The compound was identified as myricyl alcohol.

Compound D, eluted with benzene : chloroform (3 : 1), had m.p. 218° (from methanol, 80 mg),  $[\alpha]_D + 20^\circ$ ;  $\nu_{\max}$  3500  $\text{cm}^{-1}$  (OH). It formed an acetate, m.p. 215°.  $[\alpha]_D + 50^\circ$ . Liebermann-Burchard test was positive. The compound was identified as lupeol.

Compound E, eluted with benzene : chloroform (1 : 3), had m.p. 136–137° (from methanol, 200 mg),  $[\alpha]_D - 40^\circ$ ;  $\nu_{\max}$  3500  $\text{cm}^{-1}$  (OH) and gave an acetate, m.p. 125–126° (from methanol),  $[\alpha]_D - 35^\circ$ . Liebermann-Burchard and TNM tests were positive. The compound was identified as  $\beta$ -sitosterol.

Compound F, eluted with chloroform : methanol (95 : 5), did not melt up to 300° and was not



easily soluble in ordinary solvents (50 mg).  $[\alpha]_D^{25} - 50^\circ$  (pyridine). Acid hydrolysis gave  $\beta$ -sitosterol and D-glucose. Permethylation of compound F by Hakomori's method and subsequent hydrolysis by Kiliani's reagent (HCl : AcOH :  $H_2O$  : 1 : 3 : 5 : 5 : 5) gave  $\beta$ -sitosterol and 2, 3, 4, 6-tetra-O-methyl D-glucose. Enzymatic hydrolysis with emulsin showed  $\beta$ -linkage. The compound was identified as  $\beta$ -sitosterol- $\beta$ -D-glucoside.

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## METABOLISM IN *MYTILOPSIS SALLEI* (RECLUZ) (PELECYPODA): INFLUENCE OF TEMPERATURE

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THE inter-tidal zone experiences to a great degree the vicissitudes of terrestrial climate and at low tide zone the marine animals are subjected to a wide range of temperature fluctuations. Ghiretti<sup>1</sup> observed that the metabolic rate is generally related to temperature in molluscs as in other poikilotherms. The effect of temperature on the respiration of many molluscs has been studied earlier<sup>2-8</sup>. However, studies on the effect of temperature on bivalves appear to have received limited attention<sup>6-9</sup>.

*Mytilopsis sallei* (Recluz) is a central American species which has migrated to Indian waters in recent years and has shown extensive propagation in local waters<sup>10</sup>. The animal has been found to withstand variations in salinities ranging from fresh water to 50‰ as determined earlier<sup>11</sup>. In the present studies, investigations were undertaken to examine the influence of temperature ranging from 5°C to 40°C on the metabolism of the bivalve *M. sallei*.

### MATERIAL AND METHODS

The experimental animals were collected from test panels exposed at the local harbour and were allowed to acclimatise overnight in the laboratory. Healthy animals of various size groups were then selected and taken in respiratory chambers individually<sup>12</sup>. The experiments on the respiration of the animals were conducted at various controlled temperatures ranging from 5°C to 40°C at every five degree interval. It was ensured that the sea-water temperature in the experimental jars was brought to the required level prior to the beginning of the experiment. The respiratory chambers containing individual animals were then flushed with nitrogen to remove any oxygen. A series of experiments were conducted at each temperature

and the minimal and maximal rates of respiration were determined. This was regarded as the index of active and standard rates of metabolism<sup>13</sup>. Fresh lot of animals previously acclimatised in the laboratory were selected for each temperature.

### RESULTS

The metabolic rate was calculated from the following formula<sup>14</sup>:

$$Y' = aX^{b'}$$

where  $Y'$  = the respiration rate ( $Y/X$ )

$X$  = the body weight

$a$  = constant

and  $b'$  = the specific exponent of weight ( $b-1$ ).

A calculated regression line of the metabolic rates (both standard and active) in relation to body size has been drawn for each temperature level on a double log scale as shown in Fig. 1. The effect of temperature on animals of 100 mg body weight has been reconstructed from Fig. 1 and drawn on a semilogarithmic scale (Fig. 2). On the basis of standard and active metabolic rates determined earlier, scope of activity at different temperatures examined has also been determined (Fig. 3).

It may be observed from the results (Fig. 1) that the value of  $b$  varies with temperature in the mussel *M. sallei*. The slope of regression correspondingly varied with temperature both for active and standard metabolic rate. The standard rate of metabolism appeared to show a general trend, increasing with increase in temperature. The active rate, on the other hand, showed a variable response to temperature and apparently no general pattern could be observed. The active rate was generally higher at 5°C, 15°C and 30°C. The active rate of metabolism, however, decreased beyond 30°C. The scope of activity as shown in Fig. 3 indicates clearly that the maximum activity of these species is recorded at 15°C. Although another peak was

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also obtained at 30° C. the scope here was much less than that of 15° C.

by temperature changes. The basal rate of metabolism, however, showed a trend towards an increase

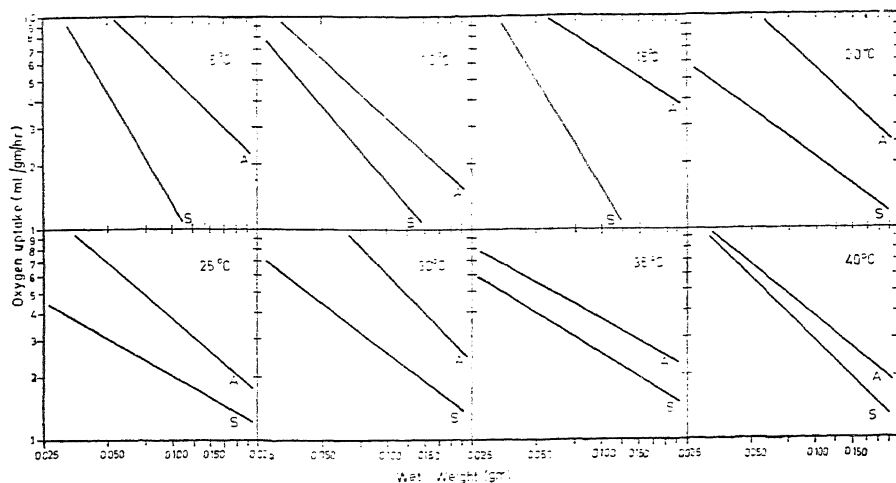


FIG. 1. Active (A) and standard (S) metabolic rates of *Mytilopsis sallei* in relation to body size at different temperatures.

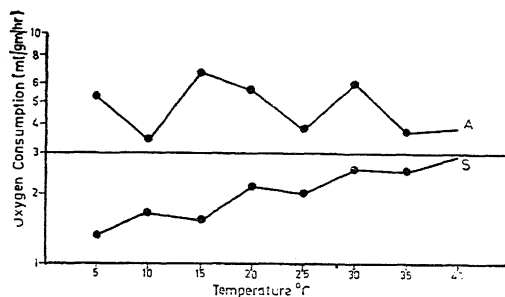


FIG. 2. The effect of temperature on the 100 mg size animal—reconstructed.

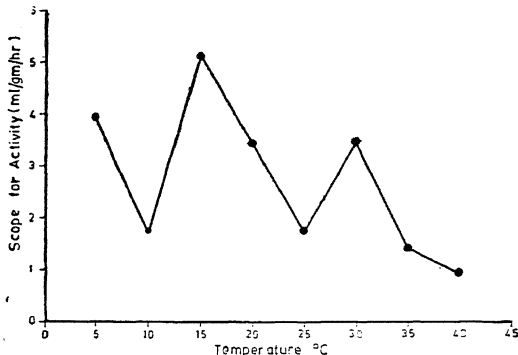


FIG. 3. Influence of temperature on the scope for activity.

#### DISCUSSION

It may be observed from the results that the metabolism in *M. sallei* remains generally unaffected

with increase in temperature. The active rate on the other hand showed fairly wide fluctuations at different temperature. Similar fluctuations in the active rate of metabolism have also been recorded earlier for *Mytilus edulis* and *Littorina littorea*<sup>9</sup>.

The effect of temperature on active and standard rates of respiration of a wide variety of inter-tidal invertebrates have been studied earlier<sup>13-15</sup> and extensively reviewed by Newell<sup>16</sup>. In many species the temperature affected the active rate of oxygen consumption to a much greater extent than the standard rate and the rate of active metabolism increased with increase in temperature upto a certain level<sup>18,17</sup>. Barcroft<sup>18</sup> and Bullock<sup>19</sup> on the other hand have demonstrated that the metabolism does not increase regularly with temperature. The scope of activity, being the difference between the active and standard rate, does not appear to follow any uniform pattern in the case of *M. sallei* and in fact is comparatively more at lower temperatures than at higher temperatures.

The significance of this temperature-independent metabolism has been discussed in detail by Barcroft<sup>18</sup>, Bullock<sup>19</sup>, Davies<sup>14</sup> and recently by Newell and Northcroft<sup>13</sup>. It is generally agreed that the relatively undisturbed metabolism over a range of temperatures would indicate an important homeostatic mechanism in a poikilotherm and that this would allow the rates of metabolic reactions to proceed at a relatively constant rate despite the fluctuations in environmental temperature in the intertidal zone.

The authors wish to acknowledge the encouragement given to this work by Captain P. R. Sen. I.N., Director and Sri. S. V. S. Rao, Deputy Director of this laboratory.

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## EFFECT OF IONIZING RADIATION ON SEED GERMINATION OF *PASSIFLORA* SPECIES

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THE recent popularity of the genus *Passiflora*, because of its edible species, has attracted the attention not only of taxonomists but also of cytogeneticists. Of the 400 known species of *Passiflora*, about 50 to 60 bear edible fruits. Probably all these are indigenous to the American tropics. In most areas of the tropical and subtropical world where passion fruit is grown, the species *P. edulis* Sims. (purple passion fruit) predominates. Purple passion fruit, because of its high fruit quality is by far more popular in Australia, New Zealand, Brazil and S. Africa. In India it is known only in some parts. Besides the juice, purple passion fruit also contains vitamin A and niacin<sup>1</sup>. Commercial production of this fruit has limitations because of its susceptibility to common pests and diseases and intolerance to cold.

Seed germination percentage has been found to be very poor in *P. edulis* and to a certain extent in *P. foetida* Linn. Ionizing radiations could be of great importance as a means for removing the dormancy of the plant material, increasing the germination and germination energy of seeds, tubers and roots.

Seeds of *P. edulis* were obtained from Sims Park, Nilgiris and those of *P. foetida* were obtained from Agriculture and Fisheries Department, Kowloon, Hong Kong. Seeds of both the species were surface sterilized before they were dispatched to BARC, Trombay, for irradiation. The irradiation treatment consisted of 12 levels of gamma rays ranging

from 1 kr to 30 kr with 150 seed sample used per dose. The entire experiment was repeated twice and the mean was tabulated and given in Table I.

TABLE I  
Germination and survival percentage of *P. edulis* and *P. foetida* seeds irradiated with gamma rays.  
Data taken after 30 days of survival

| Dose    | <i>P. edulis</i> |            | <i>P. foetida</i> |            |
|---------|------------------|------------|-------------------|------------|
|         | Germination %    | Survival % | Germination %     | Survival % |
| Control | 33.4             | 33.5       | 38.7              | 67.3       |
| 1 kr    | 87.6             | 90.4       | 80.5              | 94.6       |
| 1.5 kr  | 82.3             | 100.0      | 84.8              | 92.3       |
| 2 kr    | 88.7             | 92.4       | 81.4              | 93.4       |
| 2.5 kr  | 80.7             | 83.7       | 94.5              | 91.7       |
| 5 kr    | 74.6             | 76.8       | 89.7              | 84.7       |
| 7.5 kr  | 68.3             | 61.3       | 84.5              | 82.3       |
| 10 kr   | 59.7             | 45.7       | 79.8              | 68.4       |
| 12.5 kr | 48.3             | 43.7       | 69.4              | 58.7       |
| 15 kr   | 41.5             | 40.2       | 59.8              | 51.3       |
| 20 kr   | 18.4             | 14.8       | 33.6              | 27.8       |
| 25 kr   | 06.7             | 04.5       | 27.6              | 18.5       |
| 30 kr   | 00.0             | 00.0       | 00.0              | 00.0       |

The irradiated seeds were sown within 24 hours, in pots together with the control seeds. Survival percentage for each dose was determined.

Table I indicates that the percentage of seed germination in both the species was found to have increased at lower doses over those of control but the survival percentage of seedlings increased considerably at lower doses ranging from 1 kr to

5 kr in *P. edulis* and from 1 kr to 10 kr in *P. foetida*. After 15 kr there was observed a sudden drop in the germination as well as in the survival percentage in both the species. More or less similar type of results were obtained in *Lupinus luteus*, *Papaver somniferum*<sup>4</sup>; *Datura stramonium*<sup>5</sup>.

The seeds of both the species which were irradiated at lower doses were found to have germinated earlier than those of the control and of higher doses. The effect of ionizing radiation on the acceleration of seed germination was indicated very clearly. Between 1 kr to 2.5 kr, seeds germinated within 18 to 21 days while seeds of control *P. edulis* took 29 days for germination. While at 1 kr to 2 kr seeds of *P. foetida* germinated within 10 days but the germination was observed on 15th day in control seeds. Almost every investigator found that germination and germination energy of seeds increased after irradiation with small doses<sup>6,7</sup>.

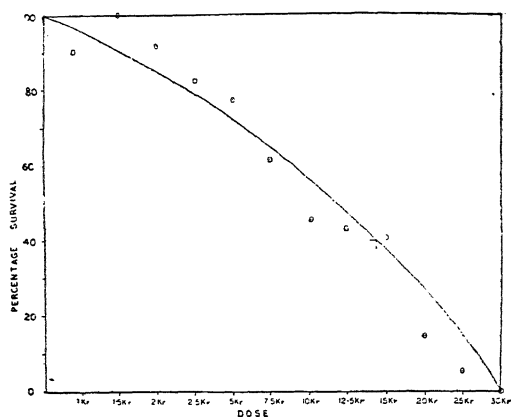


FIG. 1. Relation of survival in the seeds of *P. edulis* exposed to different doses of gamma rays.

In Sweden and in U.S.A. plant breeders who use radiation are employing the so-called "critical dose" in which about 40% of the plants survive. According to Dubinin<sup>8</sup> a critical dose of radiation is the one in which an appreciable depression is observed in the development of plants, but at which, an adequate number of plants remains capable of producing seeds. Also there is a relation between the radiosensitivity, number of chromosomes in cells

and their size in different species of plants. Such a comparison was made by Sparrow<sup>9</sup>. It has been found that in general plants with a similar number of chromosomes have almost equal radiosensitivity. The radiosensitivity is being figured here in the term of critical dose. In the present investigation *P. edulis* which has  $2n = 18$  and with large chromosomes (range  $47.9 \mu - 13.19 \mu$ ), the critical dose is 14.5 kr (Fig. 1) while *P. foetida* with  $2n = 22$  and with comparatively smaller chromosomes (range  $28.42 \mu - 4.9 \mu$ ) has a critical dose as 18 kr (Fig. 2).

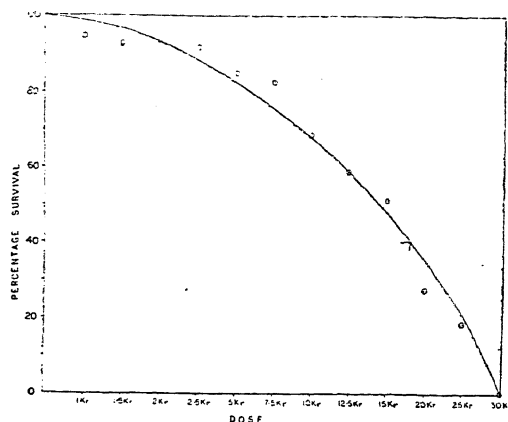


FIG. 2. Relation of survival in the seeds of *P. foetida* exposed to different doses of gamma rays.

The authors are very much thankful to the Industries Commissioner, SIRC, Government of Maharashtra, Bombay, for financial assistance.

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## EXCLUSIVELY "RED" FLIGHT MUSCLE: METABOLIC SPECIALIZATION AND SPECIFIC FLIGHT PHYSIOLOGY

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### ABSTRACT

The main flight muscle of certain bats and finches is composed exclusively of red fibres. These fibres, unlike the fibres of most mixed muscles, possess distinct capacity for glycolytic (anaerobic) as well as lipoxidative (aerobic) types of metabolism. The metabolic quality of this highly active muscle seems to be related to its contractile physiology.

### INTRODUCTION

THE flight muscles of most birds and bats, unlike other vertebrate skeletal muscles, are adapted for contractions at a high frequency for prolonged periods, resulting in their specialized histophysiological and biochemical organization. Of the two main types of fibres in these muscles<sup>1</sup>, the smaller red fibres are rich in myoglobin, mitochondrial substance, certain lipolytic and oxidative enzymes (lipoxidative), and function largely aerobically-metabolizing lipids as their chief energy fuel. The larger white fibres are poor in myoglobin, mitochondrial content, lipoxidative enzymes, rich in glycolytic enzymes, and function largely anaerobically-using glycogen as their major energy substrate. The red fibres perform slow and sustained contractions, while the white fibres are capable of relatively faster/short-lasting contractions. Certain birds and bats have been described as having only red fibres in their major flight muscle<sup>1</sup>. The metabolism of these red muscles has been closely identified with that of the usual red fibres of other muscles, as mentioned above. However, during our recent studies<sup>2</sup> on the exclusively red breast muscle of certain microchiropteran bats and weaver finches, we observed significant metabolic differences between the fibres of this muscle and the red fibres of the mixed muscles (having both red and white fibre types). Our observations are at variance with the usual metabolic speciation of the red fibres as predominantly fat-utilizing, aerobic components of muscle metabolism. We report results that suggest the distinct metabolic capacity of the pure red flight muscles to meet their energy requirements through both glycolytic and lipoxidative metabolic channels, quite unlike the fibres of other skeletal muscles. A possible correlation of such metabolic duality in these muscles with the specific flight physiology of the animals has been attempted.

### MATERIALS AND METHODS

The main flight muscle (m. pectoralis major) of certain microchiropteran bats (*Pipistrellus* sp.) and weaver finches (*Ploceus philippinus*) was used in the present study. Cryostat-cut 10  $\mu$  sections of

the fresh frozen muscle were processed for the histochemical demonstration of succinic dehydrogenase (SDH), lactic dehydrogenase (LDH),  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GPDH), phosphorylase, lipids, glycogen<sup>3</sup>, and lipase<sup>4</sup>.

### OBSERVATIONS AND DISCUSSION

Cytologically, the fibres of the pectoralis muscle could readily be identified as the usual red ones<sup>2</sup> on the basis of their mitochondrial density—apparent in the preparation for SDH activity (Fig. 1 A)—and high myoglobin content, imparting an intense red colour to the muscle. Histochemical preparations revealed the presence of both glycolytic (Fig. 1 C, D) and lipoxidative (Fig. 1 A, B) metabolic components, and also that of lipids, glycogen and LDH activity<sup>2</sup>, uniformly in all the muscle fibres. Due to this histochemical uniformity it was not possible to differentiate the muscle fibres into specifically glycolytic and lipoxidative types, that are observed in mixed skeletal muscles.

Higher level of glycogen has been reported in the red than in white fibres of certain mammalian and avian skeletal muscles<sup>5-6</sup>. Hexokinase activity has also been reported<sup>7</sup> to be higher in red than in white muscle. Besides these, *in vitro* studies on the carbohydrate metabolism in predominantly red muscles have revealed their glycolytic capacity<sup>8-10</sup>. Yet, there seems to have been no attempt to investigate critically the glycolytic capacity of totally red skeletal muscles. In the house sparrow pectoralis, presence of glycogen and phosphorylase activity has been described in the fibres of the superficial region, while the fibres of the deeper region were found to be predominantly of oxidative type<sup>11</sup>. This, in a morphologically homogeneous red muscle both glycolytic and lipoxidative activities were demonstrated (though in different regions)—in direct relation to the specific mode of flight of the animal. Closely parallel to this are our observations. Though there is no distinction of fibres (and of their regional distribution) into glycolytic and lipoxidative populations, both types of metabolic components were present uniformly in all the fibres of bat (Fig. 1) and finch pectoralis. These fibres also showed uniform myosin-adenosine triphosphatase (m-ATPase) activity throughout the

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muscle<sup>2</sup>, reflecting their identical pattern of contractility, since the m-ATPase activity of the muscle fibres is directly related to the speed of their contraction<sup>12</sup>. We have also observed that the intensity of the glycolytic activity in the fibres of the 'red' pectoralis of bats and finches is almost as high as in the white fibres of the typical skeletal muscle<sup>2</sup>.

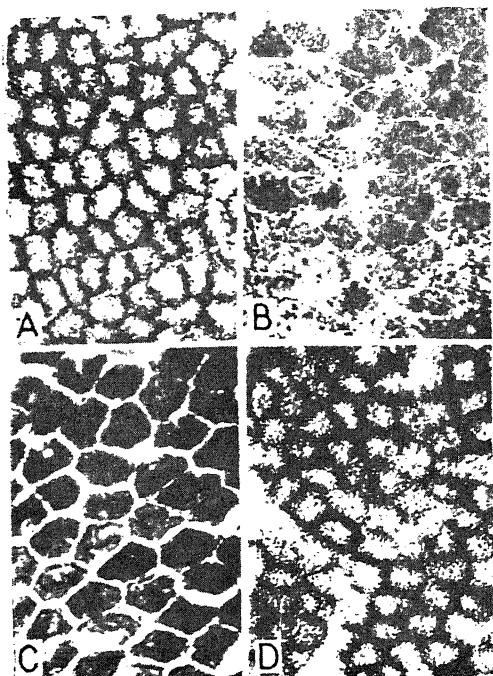


FIG. 1. Histochemical demonstration of the enzymic components of glycolytic and lipoxidative metabolism in the major flight muscle (m. pectoralis major) of the microchiropteran bat. Both lipoxidative and glycolytic enzymes are shown to have uniform activity in all the fibres, which are all of one type. (A), succinate dehydrogenase activity; (B), (C), (D), activity of lipase, phosphorylase, and  $\alpha$ -glycerophosphate dehydrogenase respectively,  $\times 200$ .

Slow and fast contractions, in the pectoralis of most birds and bats, are the individual functions of red and white fibres—using mainly lipids and glycogen respectively. Thus, it seems only appropriate to elicit from our observations that the occurrence of both glycolytic and lipoxidative metabolic components uniformly in all the individual fibres of the 'red' pectoralis of the small microchiropteran bats and weaver finches (probably also in other birds having similar pectoralis) is an essential parallel of their capacity to perform both slow and fast contractions during flight. Physiological studies, using electromyographic recordings of the pectoralis muscle (composed exclusively of red fibres) of certain birds, have revealed the capacity of such 'red' fibres to perform both slow and fast contractions during flight<sup>13-15</sup>. We suggest that a physio-

logical shift from a fast to slow contraction (or vice versa) during flight is accompanied by a metabolic shift at cellular level—of which these 'red' fibres are quite capable since they possess both glycolytic and lipoxidative metabolic systems. Similar metabolic shift has been reported in dogs—where longer periods of muscular exercise showed gradual shift from carbohydrate to fat utilization<sup>16</sup>. It is likely that in the exclusively red pectoralis of bats and finches, energy derived from glycolysis is used during the short bursts of flight (requiring instant energy), while that from lipoxidative activity is utilized for relatively sustained flight. This interpretation however can be substantiated only by comparing the metabolic profile of the muscle at rest, as in the present study, with that of the muscle immediately after flight.

This, to our knowledge, is the first suggestion of the possible operation of both glycolytic and lipoxidative metabolic cycles within the same individual fibres of the exclusively red pectoralis of the active fliers. Evidently, a pure red muscle also possesses the metabolic equipment that is normally, and almost exclusively, characteristic of the white fibres of mixed skeletal muscles, suggesting thereby a local metabolic specialization of the muscle fibres in response to the specific flight physiology of the animals.

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## LETTERS TO THE EDITOR

EFFECT OF MAGNETIC ACTIVITY ON NOON  
BITE-OUT AT KODAIKANAL—INFLUENCE  
OF SOLAR ACTIVITY

THE occurrence of a prominent trough accompanied by two maxima, one in the forenoon and the other in the afternoon in the diurnal variation of foF2 at equatorial latitudes referred to as noon 'bite-out', is a characteristic feature at stations that fall in the trough of the 'equatorial anomaly'. (Appleton, 1946; Bailey, 1948; Maeda, 1955; Rastogi, 1959). Sarma and Mitra (1956) studied the noon bite-out phenomenon by introducing two parameters:  $P_1 = (f_1 - f_2)/f_2$  and  $(f_3 - f_2)/f_2$ , where  $f_1$ ,  $f_2$  and  $f_3$  are the frequencies corresponding to the forenoon peak, midday trough and afternoon peak respectively in the diurnal variation of foF2. The ratio  $P_1/P_2$  is thus an index of the asymmetry in the noon bite-out effect. It was shown by Sarma and Mitra (1956) that the ratio  $P_1/P_2$  attains a value of unity at a particular sunspot number known as critical sunspot number which is found to increase with increase in magnetic dip. A later study by Raju and Rao (1959) for a number of equatorial stations indicated the critical sunspot number to increase with decrease in  $|L-I|$  where L and I are the geographic latitude and magnetic dip respectively. Bhargava and Subrahmanyam (1962) studied the influence of disturbed geomagnetic conditions on the noon-bite-out in foF2 at Kodaikanal for the period 1955–58 and reported the midday trough and the afternoon peak in foF2 to be reduced during disturbed conditions compared to quiet conditions, while the forenoon peak is more or less unaffected. This means the ratio  $P_1/P_2$  is high during disturbed conditions compared to quiet conditions.

In the present investigation, the effect of disturbed geomagnetic conditions on the asymmetry of the bite-out phenomenon at Kodaikanal (Geo. Mag, Lat.  $0.6^\circ$  N, Dip:  $3.5^\circ$  N) over the ascending phase of the solar cycle (1964–69) has been studied to infer the influence of solar activity. Published monthly ionospheric data of Kodaikanal for the period 1964–69 has been used for this purpose. For each month, the data have been divided into those of quiet conditions ( $\Sigma K_p \leq 10$ ) and disturbed conditions ( $\Sigma K_p \geq 25$ ) where  $K_p$  is the planetary K-index of geomagnetic activity. The median values of foF2 at half-an-hour intervals were calculated for both the periods separately from which the parameters  $P_1$ ,  $P_2$  and  $P_1/P_2$  have been evaluated. Median values of sunspot number corresponding to

the two periods have also been obtained for each month. Running averages were then calculated to smooth out short term and seasonal variations.

In Fig. 1 we show the variation of the ratio  $P_1/P_2$  with sunspot number for the quiet and disturbed periods separately. The following points may be

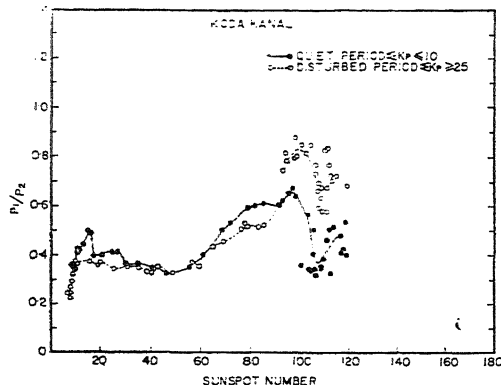


FIG. 1. Variation of  $P_1/P_2$  with sunspot number for quiet and disturbed conditions at Kodaikanal for the period 1964–69.

noticed. Although the ratio  $P_1/P_2$  shows a general tendency to increase with sunspot number, its behaviour during low and moderate solar activity periods is different in that it increases with sunspot number during low solar activity periods ( $R_s < 20$ ) and decreases during moderate solar activity periods ( $R_s > 100$ ). Further, the ratio  $P_1/P_2$  is high during magnetically disturbed conditions compared to quiet conditions during the periods of moderate solar activity ( $R_s > 100$ ), while the behaviour is exactly opposite during the periods of low solar activity ( $R_s < 20$ ) as can be clearly seen from Fig. 1. These observations are considered to be interesting in view of the following considerations.

The bite-out phenomenon is obviously the result of interaction and the relative role played by the production, loss and movements terms in the continuity equation of the electron density at the peak of the layer. The movements term includes the effects of vertical drift, horizontal diffusion of ionization along the field lines and neutral winds. Martyn (1955) and Rao (1967) showed that the forenoon peak in the diurnal variation of foF2 at equatorial latitudes is influenced by horizontal winds besides production and loss processes, while the afternoon peak is determined by vertical drifts and diffusion (Gliddon and Kendall, 1962). Since the

results of Bhargava and Subrahmanyam (1962) indicate that the forenoon peak is not much affected by magnetic activity, an attempt will now be made to account for the results of the present study, on a qualitative basis, in terms of vertical drifts, and horizontal diffusion besides production and loss processes. It is known that during disturbed conditions when the horizontal component of the magnetic field is subnormal, there will be a considerable lowering of the height of the layer due to vertical downward drift of plasma (effect of westward electric currents). As such, during periods of low solar activity when the vertical movements play a relatively important role, there should be considerable inhibition of loss of ionization due to lowering of the layer resulting in high values of  $P_1/P_2$  for disturbed conditions compared to quiet conditions, while there is not to be much of a difference between the values of  $P_1/P_2$  for disturbed and quiet conditions during relatively high solar activity periods. The observed result is exactly opposite to that expected on the conceived simplified picture of the origin of the noon bite-out phenomenon.

To sum up, the present investigation revealed a significant influence of solar activity on the relative trend in asymmetry in noon bite-out during quiet and disturbed conditions. This feature does not facilitate even a qualitative understanding in terms of vertical upward drift and subsequent horizontal diffusion of ionization along the field lines.

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## LEVEL STRUCTURE OF $^{199}\text{Hg}$

DE-SHALIT<sup>1</sup> tested the applicability of the core-excitation model to  $^{199}\text{Hg}$  using the then available data and reported that the consistency of the model with regard to the magnetic moment and the transition rates are still to be tested. Hence in the present note the applicability of the core-excitation model to all the electromagnetic properties of first two excited levels is tested. In addition the properties are also compared with the estimations based on single particle as well as Kisslinger and Sorensen models<sup>2-4</sup>. Details of the calculations are given elsewhere<sup>5-7</sup>.

The core-excitation model in its simplest version envisages the levels in  $^{199}\text{Hg}$  as arising from the coupling of a  $p_{3/2}$  neutron to the first  $2^+$  state of the  $^{198}\text{Hg}$  core. In these calculations recent experimental data are taken<sup>8-10</sup>. The following parameters gave the best fit :

$$g_p = 0.75 \text{ [Schmidt value, } \mu/\sqrt{I(I+1)}]$$

$$A^2 = 0.988$$

$$g_c = 0.4 \text{ (= } Z/A \text{) and}$$

$\langle O(T_c^2) \rangle = 0.179$  (average value of  $^{198}\text{Hg}$  and  $^{200}\text{Hg}$ ). The value of  $A^2$  is very close to 1 in conformity with the requirement of the core-excitation model. The results are tabulated in Table I.

As can be seen from Table I, an excellent agreement is obtained between the predictions of core-excitation model and the corresponding experimental

TABLE I  
Comparison between theory and experiment

| Description   | Experiment           | Core-excitation | Single particle | Kisslinger et al. <sup>9</sup> |
|---|----------------------|-----------------|-----------------|--------------------------------|
| B (M1) $3/2 \rightarrow 1/2$ in units of $eh/2mc^2$ (208 keV)                         | 0.082<br>$\pm 0.016$ | 0.082           | 7.0             | ..                             |
| B (M1) $3/2 \rightarrow 5/2$ in units of $eh/2mc^2$ (50 keV)                          | 0.028<br>$\pm 0.003$ | 0.074           | 12.6            | ..                             |
| B (E2) $5/2 \rightarrow 1/2$ in units of $e^2 \times 10^{-48} \text{ cm}^4$ (158 keV) | 0.175<br>$\pm 0.007$ | 0.179           | 0.0069          | 0.053                          |
| B (E2) $3/2 \rightarrow 1/2$ in units of $e^2 \times 10^{-48} \text{ cm}^4$ (208 keV) | 0.08<br>$\pm 0.016$  | 0.17            | 0.0069          | 0.0088                         |
| $\mu_{1/2}$ in units of n.m.  | 0.5                  | 0.43            | 0.423           | 0.32                           |
| $\mu_{5/2}$ in units of n.m.  | 1.03<br>$\pm 0.08$   | 1.25            | 2.12            |                                |
| $\mu_{3/2}$ in units of n.m.  | ..                   | 0.448           | 1.27            |                                |

values. From the present work it may be concluded that the level structure of  $^{199}\text{Hg}$  can be satisfactorily



explained within the framework of the core-excitation model.

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### A STUDY OF MOLECULAR FORCE FIELD ELLIPSES FOR $\text{SnCl}_2$ WITH LONE-PAIR

The stannous dichloride is a typical example of  $\text{XY}_2$  type of molecules and is of particular importance in the studies of lone-pair Chemistry. Considerable information on the vibrational frequency data of  $\text{SnCl}_2$  is available in the literature<sup>1,2</sup>. The object of the present note is to report the investigations made by us on the molecular vibrations of  $\text{SnCl}_2$  based on Green's function and Partitioning (GFP) techniques<sup>3</sup>.

The method used, which is reported earlier<sup>4-6</sup>, involves the following product and sum rules of frequencies for the isotopic substitution  $\text{XY}_2\text{L} \rightarrow \text{XY}_2^*\text{L}$  for  $\text{SnCl}_2$ , with lone-pair electron contributions:

$$\omega_1^{*2} \omega_2^{*2} = \left( \frac{\sigma}{\alpha^2 \epsilon - \sigma} \right) \left( \frac{m_y}{m_y^*} \right)^2 \omega_1^2 \omega_2^2 \quad (1)$$

$$\omega_1^{*2} + \omega_2^{*2} = \left[ \frac{\sigma C^2 + \alpha^2 \epsilon}{C^2 (\alpha^2 \epsilon - \sigma)} \right] \left( \frac{m_y}{m_y^*} \right) \omega_1^2 + \left[ 1 - \frac{\alpha^2 \epsilon}{C^2 (\alpha^2 \epsilon - \sigma)} \right] \left( \frac{m_y}{m_y^*} \right) \omega_2^2 \quad (2)$$

$$\epsilon = \frac{m_y - m_y^*}{m_y^*}, \quad \sigma = \frac{m_y^* m_x \epsilon}{m_y d_1^2} + 1,$$

$$\alpha = \frac{\sqrt{2 m_y m_y^{*3/2}}}{[m_y^* m_x - m_e m_y] d_s}$$

$$C^2 = 1 - c^2, \quad d_1^2 = \frac{1}{m_y} [m_x m_y^4 - 2 m_y m_y^* + m_x m_e]$$

$$d_s = \left[ \frac{m_y^* m_x}{m_x m_y^* - m_y m_e} \right]^{1/2} d_1$$

The experimental frequency assignment for  $\text{SnCl}_2$  is ambiguous in fixing the valence angle and deciding whether  $\omega_1 > \omega_2$  or *vice versa*. Our study on  $\text{SnCl}_2$  reveals that the mixing parameter becomes imaginary for the case  $\omega_1 < \omega_2$ , unless  $\omega_1^*$  has a frequency assignment in the range  $325 \text{ cm}^{-1}$  to  $329 \text{ cm}^{-1}$ , but then this value of  $\omega_1^*$  is not correct as per the IR spectra reported by Andrews *et al.*<sup>7</sup>. Hence we conclude  $\omega_1 > \omega_2$ , which is in agreement with the conclusion drawn from the electronic spectrum of  $\text{SnCl}_2$  by Naegeli *et al.*<sup>11</sup>.

The molecular force field ellipses for  $\text{SnCl}_2$  have been obtained for valence angles  $90^\circ$ ,  $95^\circ$ ,  $100^\circ$  and  $120^\circ$  for (1)  $\omega_1 = 354.8 \text{ cm}^{-1}$ ,  $\omega_2 = 120 \text{ cm}^{-1}$ ,  $\omega_3 = 334.6 \text{ cm}^{-1}$ , (2)  $\omega_1 = 334.6 \text{ cm}^{-1}$ ,  $\omega_2 = 120 \text{ cm}^{-1}$ ,  $\omega_3 = 354.8 \text{ cm}^{-1}$  and a few are given in Figs. 1 and 2. From the study of the ellipses, it is observed that for a given valence angle the lone-pair electron contribution causes the eccentricity to increase; while the angle of inclination of the ellipse with the  $F_{11}$  axis decreases considerably. This is an interesting feature which implies that the inclusion of lone-pair is as if there is an increase in the mass of the central atom and which follows from the analysis of molecular force field ellipses by Torkington<sup>10</sup>.

FIGURE 1

$$\omega_1 > \omega_3$$

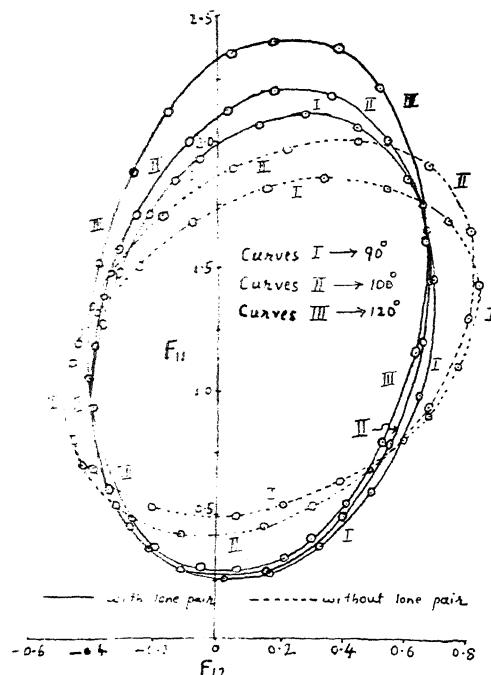


FIG. 1.  $F_{11}$  as a function of  $F_{12}$  for  $\omega_1 > \omega_3$ .

TABLE I

Symmetry mean square amplitudes, force constants and coriolis coupling coefficients for  $\text{SnCl}_2$  with lone-pair

| Symme. mean sq. amplitudes in $\text{\AA}^2$ |              |              | Force constants<br>in<br>mdyne/ $\text{\AA}$ | Coriolis coupling coefficients |              |
|--|--------------|--------------|--|--------------------------------|--------------|
| T = 0° K                                     |              | T = 298° K   |  |                                |              |
| a  | 0.001, 9336  | 0.003, 3562  | 2.115  |                                | -0.004, 4059 |
| b  | 0.001, 8380  | 0.003, 0231  | 2.154  | $\zeta_1$                      | -0.048, 8186 |
| c  | 0.001, 7535  | 0.002, 7422  | 2.186  |                                | +0.101, 5521 |
| d  | 0.000, 9788  | 0.001, 4132  | 2.238  |                                | +0.248, 0224 |
| a  | 0.015, 5552  | 0.055, 1337  | 0.113  |                                | 1.287, 720   |
| b  | 0.015, 8418  | 0.056, 1332  | 0.101  | $\zeta_2$                      | 1.280, 725   |
| c  | 0.016, 0955  | 0.056, 0760  | 0.090  |                                | 1.272, 040   |
| d  | 0.010, 7248  | 0.037, 2027  | 0.072  |                                | 0.977, 414   |
| a  | 0.001, 6827  | 0.002, 5171  | 1.973  |                                | 0.000, 0194  |
| b  | 0.001, 7300  | 0.002, 5879  | 1.919  | $\zeta_1$                      | 0.002, 3836  |
| c  | 0.001, 7791  | 0.002, 6614  | 1.866  |                                | 0.010, 3129  |
| d  | 0.001, 9710  | 0.002, 9484  | 1.684  |                                | 0.061, 5151  |
| a  | -0.002, 0475 | -0.007, 2783 | 0.279  |                                | 1.658, 2315  |
| b  | -0.001, 7980 | -0.006, 1341 | 0.234  | $\zeta_2$                      | 1.640, 2580  |
| c  | -0.001, 5291 | -0.004, 9163 | 0.186  |                                | 1.618, 0863  |
| d  | -0.001, 5291 | -0.000, 1572 | -0.014                                       |                                | 0.955, 3380  |

Note : a, b, c and d refer to bond angles 90°, 95°, 100° and 120° respectively.

FIGURE 2

$\omega_1 < \omega_3$

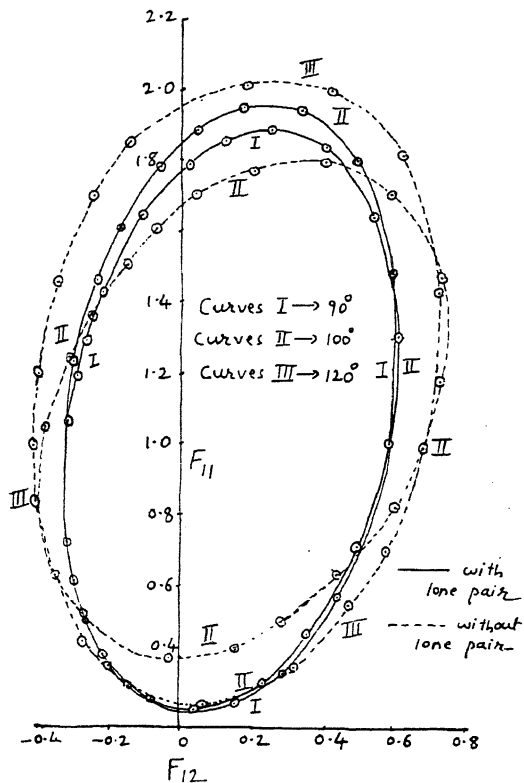


Fig. 2.  $F_{11}$  as a function of  $F_{12}$  for  $\omega_1 < \omega_3$ .

In the case  $\omega_1 > \omega_3$ , using equations (1) and (2) together with  $\omega_1^4 = 352.3 \text{ cm}^{-1}$  (reported value<sup>1</sup>), the mixing parameter comes out to be imaginary. Therefore by selecting  $\omega_1^4 = 346.5 \text{ cm}^{-1}$  (from the IR spectra<sup>1</sup>) and by varying the mixing parameter we arrive at a consistent set of molecular constants given in Table I.

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# VARIATION OF INTERNAL PRESSURE WITH TEMPERATURE IN LIQUID OXYGEN, ARGON, NITROGEN AND METHANE

RECENTLY, Suryanarayana<sup>1</sup> brought out the thermodynamic significance of internal pressure and in a private communication stated that the internal pressure should fall exponentially with increase in temperature. This aspect is verified in the present investigation by examining the data of internal pressure of oxygen, argon, nitrogen and methane in their liquid state. The method of calculation of internal pressure is based on the method given by Suryanarayana (*loc. cit.*). The equation used is

$$\pi = \frac{T\alpha}{\beta_T}$$

where  $\alpha$  is the thermal coefficient of expansion,  $\beta_T$  is isothermal compressibility and  $T$  is the absolute temperature. The data for all these liquids have been obtained from Rowlinson<sup>2</sup>. The plots of  $\ln \pi$  versus the absolute temperature are presented in the graph. As predicted by Suryanarayana (*loc. cit.*) all plots are clearly linear and  $\ln \pi$  decreases with increase of temperature.

In the case of oxygen the plot is made up of two straight lines. A very interesting observation in the case of oxygen is that in the region of 60° to 75° K, the internal pressure varies very little with temperature. Several other data of oxygen presented by Russell B. Scott<sup>3</sup> in the same range of temperatures do not give a clue to this behaviour of oxygen between 60° to 75° K. The experimental results of Kandra, Haseda and Otsubo<sup>4</sup> on the paramagnetic susceptibility of solid and liquid oxygen show no abnormality in this region.

A plot of  $\ln P_{exp}$  versus internal pressure also exhibits an abnormal trend in this range of temperature. The data of the variation of viscosity with temperature postulated by Rudenko and Shubnikov<sup>5</sup> give a similar abnormal variation of viscosity with temperature in the same range. The data on the variation of dielectric constant with temperature of Warlaw Warner and Keesom<sup>6</sup> show a similar abnormality in the same range of temperature.

The only plausible explanation seems to be that in this condensed region there seems to be some weak transient association of oxygen molecules forming the polymer  $O_4$ , which is said to be responsible for the blue colour of liquid and solid oxygen. Oxygen has the outstanding difference from most other fluids in its being strongly paramagnetic. Thus  $O_2$ ,  $O_4$  mixtures and equilibria due to different proportions of these constituents could perhaps explain an overall cancellation effect by which the internal pressure remains almost constant.

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## CONDUCTOMETRIC STUDY OF $CdI_2$ -KI SYSTEM

CONDUCTIVITY measurements by Van Rysselberghe and Nutting Lee<sup>1</sup> of mixtures containing  $CdI_2$  and KI showed large deviations from the mixture rule, suggesting ion-pair formation. Satyavati *et al.*<sup>2</sup> studied the ultrasonic velocity and adiabatic compressibility of  $CdI_2$ -KI mixtures in aqueous medium and concluded from the results that the formation of the complex ion  $CdI_4^{--}$  is most likely. Raman spectra of cadmium halides in aqueous solutions of alkali halides examined by Woodward and co-workers<sup>3</sup> have also indicated the presence of an ion-pair of the type  $CdX_4^{--}$ .

This paper describes the conductivity measurements of solutions of  $CdI_2$  and KI individually and together in aquo-organic solvents, measured under varying conditions to establish the structure of the ion-pair formed in such solutions, and the stability constant from the data thus obtained.

**Results and Discussion.**—Cadmium iodide and potassium iodide used in the experiments were of Analar grade and the solutions of electrolytes of required concentration were prepared either in conductivity water or 75% (V/V) methanol in conductivity water. The methanol (Extrapure, Merck) was distilled twice before use. Conductometric measurements were made using Toshniwal Conductivity Bridge (Model: CL 01/01) and a dip type conductivity cell. The accuracy of the instrument is  $\pm 2\%$ . All measurements were made at 30° C.

The conductivity of the mixture of  $\text{CdI}_2$  and KI was always less than the sum of the conductivities of  $\text{CdI}_2$  and KI taken separately at constant volume indicating ion-pair formation. The molar composition of the ion-pair was determined by Job's method of continuous variation<sup>4</sup>, for equimolar solutions in the concentration range 0.03 M to 0.07 M. The deviation of conductivity,  $\Delta\lambda = \lambda_1 + \lambda_2 - \lambda_3$ , (where  $\lambda_1$  is the conductivity of a series of  $\text{CdI}_2$  solutions and  $\lambda_2$  that of KI solutions,  $\lambda_3$  that of the mixture of  $\text{CdI}_2$  and KI), was plotted against the ratio  $[\text{CdI}_2]/\{[\text{CdI}_2] + [\text{KI}]\}$ . The plots obtained (Fig. 1) clearly show that the ion-pair contains one molecule of  $\text{CdI}_2$  and two molecules of KI. These are in agreement with the results of the earlier workers<sup>2,3</sup>.

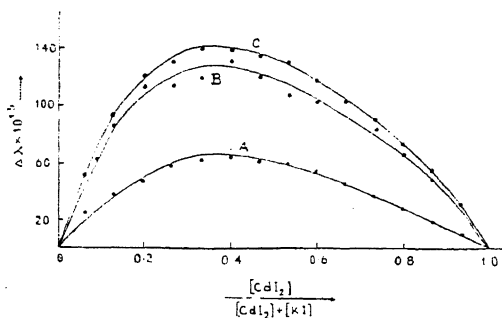


FIG. 1. Job's plot for  $\text{CdI}_2$ -KI system in aqueous medium at 30°C. Concentration of the mixture A—0.03 M; B—0.05 M; C—0.07 M.

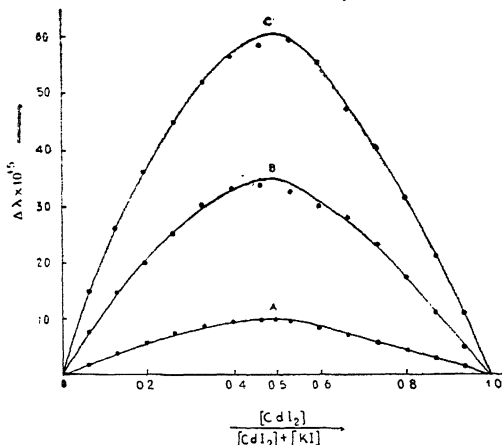
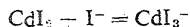


FIG. 2. Job's plot for  $\text{CdI}_2$ -KI system in 75% (v/v) methanol at 30°C. Concentration of the mixture A—0.01 M; B—0.03 M; C—0.05 M.

The above procedure was repeated in 75% (V/V) methanol solutions in water with electrolyte concentration ranging from 0.01 to 0.05 M. The data so obtained are given in Fig. 2. These curves indicate that the ion-pair contains  $\text{CdI}_2$  and KI in the ratio of 1:1. Hence, we propose that the

ion-pair formed is of the type  $\text{CdI}_3^-$ . The equilibrium may be represented as:



The stability constant of the above has been calculated by the method of Hegenmuller<sup>5</sup>. For two mixtures of molarity  $M_1$  and  $M_2$ , where the concentrations are not very different, the corresponding instability constant ( $k$ ) is given by

$$k = \frac{(M_1/2 - Z_1)^2}{Z_1} = \frac{(M_2/2 - Z_2)^2}{Z_2} \quad (1)$$

where  $Z_1$  and  $Z_2$  are the concentrations of the complexes at the maxima of the continuous variation curves in the two systems. From Equation (1) we get

$$Z_2 = \frac{M_1 - M_2}{2(a-1)} \pm \frac{aM_2 - M_1}{2(a-1)\sqrt{a}} \quad (2)$$

where  $a = Z_1/Z_2$ . Minus sign was used for  $M_2/2 > Z_2 > 0$  and  $M_1/2 > Z_1 > 0$ . Since the heights of the maxima ( $Z_1$  and  $Z_2$ ) of the continuous variation curves could be found for the two mixtures, it is possible to calculate  $a$  and hence  $Z_1$  and  $k$ . The stability constant  $K$  is therefore given by

$$K = 1/k = 1.6 \pm 0.2 \times 10^3 \text{ and } pK = -3.20 \pm 0.06.$$

From the relationship  $-\Delta G^\circ = RT \ln K$ , the free energy of formation was found to be  $-4.44 \pm 0.09 \text{ kcal.mole}^{-1}$  at 30°C.

The stability constant was also determined by extrapolation of Job's curves as suggested by Raghavarao and coworkers<sup>6</sup> using the expression,

$$K = \frac{1-a}{a^2 \cdot C}$$

where  $a$  is the degree of dissociation and 'C' is concentration. The values thus found are as follows:

$K = 2.0 \pm 0.3 \times 10^3$  and  $pK = -3.30 \pm 0.06$ . These values are in close agreement with those obtained by using Hagenmuller's method<sup>5</sup>.

The authors wish to thank Prof. N. V. Subba Rao, for his keen interest and encouragement.

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# SPECTROPHOTOMETRIC DETERMINATION OF COPPER (II) USING 1-(2-PYRIDYL-AZO)-2-PHENANTHROL (PAP) AS A CHROMOGENIC REAGENT

ANALYTICAL applications of a large number of heterocyclic azo dyes have been reviewed<sup>1,2</sup>. 1-(2-Pyridylazo)-2-phenanthrol (PAP), a chelating agent<sup>3,4</sup> forms pinkish-red coloured complex immediately on mixing with copper(II). A stock solution of PAP (0.001 M) was prepared by dissolving it in methanol. Cu(II) stock solution was prepared by dissolving  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (AnalaR, B.D.H.) in doubly distilled water and it was standardised gravimetrically. All other chemicals used were of reagent grade. 1,4-Dioxan was refluxed for about 5 hours over sodium metal and then distilled fresh before use.

A Unicam, SP-600, spectrophotometer was used for absorbance measurements and a Metrohm pH meter, E-350, for measuring pH's.

The pinkish-red, water-insoluble complex is extractable in various organic solvents, such as chloroform, carbon tetrachloride and benzene. The complex is soluble in 70% (v/v) methanol but the precipitate appears after a few minutes. To keep the complex and reagent in solution water: methanol: dioxan (35:50:15) medium was used. It was found that dioxan does not affect the absorbance of the complex even if present in larger quantities (up to 30%). The complex was found to be stable for 72 hours, after which measurements were discontinued.

The absorbance of the complex, which shows maximum at 560 nm, remains constant in the pH range 2.2–8.0 and above pH 8.0 and below pH 2.2, absorbance falls. A mole ratio study revealed that for full colour development twice the calculated amount of the reagent, PAP, is required. In subsequent work, five times molar excess of the reagent was maintained. The system obeys Beer's law up to 3.2 ppm of copper. Optimum concentration range for the determination of copper, evaluated by Ringbom's plot has been found to be 0.54 to 2.39 ppm. The sensitivity of the colour reaction (in terms of Sandell's definition) is  $0.0020 \mu\text{g Cu/cm}^2$  at 560 nm. The molar absorptivity is  $3.0 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$ . The apparent instability constant, calculated from mole ratio plot, was found to be  $8.3 \times 10^{-6}$  at room temperature. The mole ratio method, slope ratio method and Job's method of continuous variations suggest that 1:1 [Cu(II):PAP] complex is formed in the system.

Synthetic solutions containing known amounts of copper (1.08 ppm) and varying amounts of diverse

ions were prepared and copper was determined in their presence at pH 3.0. The amounts of ions and anions, given in parentheses in ppm, did not cause deviation of more than 3% in absorbance:  $\text{PO}_4^{3-}$  (160),  $\text{Cl}^-$  (1000),  $\text{Br}^-$  (800),  $\text{I}^-$  (664),  $\text{NO}_3^-$  (620),  $\text{SO}_4^{2-}$  (200), oxalate (10), tartrate (640), citrate (380), thiourea (100),  $\text{CNS}^-$  (100),  $\text{Cr}(\text{III})$  (400),  $\text{Sr}^{2+}$  (200),  $\text{Ca}^{2+}$  (200),  $\text{Ba}^{2+}$  (200),  $\text{Mn}^{2+}$  (100),  $\text{La}^{3+}$  (14),  $\text{Th}(\text{IV})$  (15),  $\text{P}(\text{IV})$  (39),  $\text{Ru}(\text{III})$  (10),  $\text{Os}(\text{VIII})$  (5). However, EDTA inhibits colour formation while  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{F}^-$ , Fe(II and III), Hg(II),  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ , V(V), U(VI),  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ , Rh(III) and Pd(II) interfere seriously. Interference due to 5 ppm each of  $\text{Cd}^{2+}$ , V(V), U(VI), Pd(II) and Rh(III) was removed by adding 50 ppm of the citrate. Mercury (10 ppm) was masked with iodide, while Fe(II and III) (5 ppm) with tartrate (50 ppm). Attempts to mask  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$  were unsuccessful.

**Recommended Procedure.**—To a suitable aliquot of solution containing between 5.4–23.9  $\mu\text{g}$  of copper, is added five times molar excess of reagent (PAP) in methanol. The pH of the solution is adjusted between 2.2–8.0 using sodium acetate-acetic acid buffer and the total volume is made to 10 ml with doubly distilled water, maintaining 50% methanol and 15% dioxan medium (to keep the complex and the reagent in solution). The absorbance is measured at 560 nm against the corresponding reagent blank and the amount of copper is deduced from the calibration curve.

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ON THE OCCURRENCE OF SOME JURASSIC  
NODOSARIOID FORAMINIFERA FROM THE  
MANFARA DOME SECTION OF THE WAGAD  
HILL BLOCKS, EASTERN KUTCH

ALTHOUGH exposures of the Mesozoic rocks have long been known from the Wagad hills of the eastern Kutch, very little has been published on its microfaunal contents. The more recent work on the Wagad rocks is that of Biswas (1971) and Deshpande (1972). Based on the stratigraphical and structural studies of this region, Deshpande (*op. cit.*) has proposed a detailed and revised stratigraphical classification. The present authors who have taken up the study of Wagad rocks with a view to investigate its microfaunal contents have followed Deshpande's classification.

The Adhoi Member of the Kanthkot Formation is exposed in the Manfara Dome section (23° 30', 70° 24') in the westernmost part of the Wagad hills. In this section, the Adhoi Member is represented by 20 meters of grey to khakhi coloured siltstones and shales. The base of this member is not exposed here, while it is overlain disconformably by a conspicuous hard calcareous sandstone band studded with megafossils of *Astrate*, *Trigonia*, *Pleuromya*, *Pholadomya*, *Pinna*, etc.

The shales and silts of Adhoi Member were processed in the laboratory and a study of its microfaunal assemblage has revealed twelve genera and nineteen species of *Nodosarioid* foraminifera.

This foraminiferal assemblage is being reported by the authors for the first time. The fossils recognised and identified are as under:

*Robulus* sp.; *R. Carinocordatus* (Subottina and Srivastava); *R. stephensoni* (Cushman); *Lenticulina dilectaformis* (Subottina and Srivastava); *L. navicula* (d' Orbigny); *Palmula* sp.; *Astacolus calliopsis* (Reuss); *A. renominata* (Schwager); *A. centrogyrata* (Terquem); *Saracenaria* sp.; *Marginulina* sp.; *Vaginulinopsis hybrida* (Terquem); *Dentalina gracilis* (d' Orbigny); *Nodosaria* sp.; *Nodosaria affinis* (Reuss); *Rectoglandulina oviformis* (Terquem); *Pseudoglandulina* sp.; *Lagena* sp.; *Lagena hispida* (Reuss).

In the above assemblage, various species of *Robulus* constitute 36%, *Astacolus* 13%, *Rectoglandulina* 11% and *Lagena* 10%; while the rest is made up of the remaining *Nodosarioid* species. In addition to these forms, a few species of Ostracodes and some doubtful Bryozoans have also been noted. On the whole, ostracodes present less varieties of species than the *Nodosarioid* foraminifera.

The most striking features of Manfara dome microfauna are: (i) the occurrence of several species of a single family of *Nodosariidae*, (ii) forms with radiate, glassy, calcareous indistinctly trans-

parent shell material often resembling milk glass—characteristic of the Triassic and Jurassic *Nodosarioid* foraminifera which Brotzen (1963) prefers to call the "mesonodosarioid" stage in the *Nodosarioid* evolution, and (iii) a general absence of other foraminiferal families.

Some of these species and genera along with many other foraminiferal families have been reported from the other localities of Kutch Mesozoic (Agrawal and Singh, 1961) from the exposures of Habo series at Walkhawas Tank and Fakirwari village and by Subottina *et al.* (1960) from the Lodia village and Khawada.

Based on their studies of these fossils, the authors have proposed an Upper Callovian-Oxfordian age to the siltstone-shaly facies of the Adhoi Member of the Manfara Dome section.

Paleoecology of these fossils is rather difficult to interpret as very little is known about the ecology, living habits and the physiology of their living representatives.

General absence or the lack of development of other foraminiferal families in this area needs further investigation. A detailed study of this area is under progress and more exhaustive description of these forms along with biostratigraphical and paleoecological interpretations will be published in due course.

The authors are thankful to Professor S. S. Merh for many useful discussions and a critical scrutiny of the text of this paper. They also gratefully acknowledge the help rendered by Dr. S. V. Deshpande of O.N.G.C.

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CHONDRITES, STERNBERG, A TRACE FOSSIL  
FROM THE DALMIAPURAM FORMATION,  
TRICHINOPOLY DISTRICT, S. INDIA

PRESENT note records the first ever find of *Chondrites* Sternberg in India. It is collected from the Grey Shale horizon in the Dalmiapuram Formation<sup>1-3-9</sup> near Kallakudi.

Genus *Chondrites* Sternberg, 1833 (non McCoy  
1848)  
*Chondrites* sp.

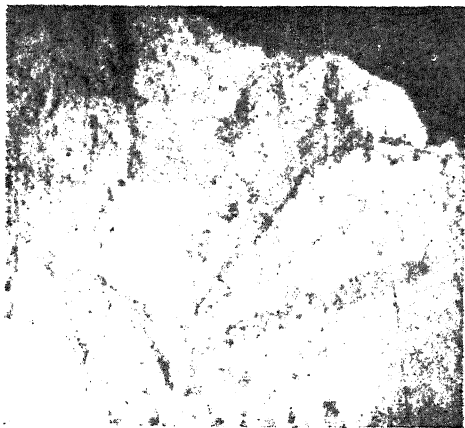


FIG. 1. *Chondrites* sp.,  $\times 1$ .

**Material :** 3 slabs ; figured specimen No. K1 8/72.  
**Dimensions :** Diameter of tunnel 2 mm.

**Description :** These are feeding burrows with plant-like branching tunnels of constant diameter (2 mm) and a circular cross section. Tunnels are filled partly with loose grey sand particles, which but for a darker shade, are like the surrounding matrix, and partly with pellet-like grains more or less transverse to the tunnel length and may be the faecal pellets as mentioned by Häntzschel<sup>5</sup>.

**Remarks :** Much smaller diameter distinguishes this species from *Chondrites* sp. from the Upper Cretaceous of Austria as figured by Häntzschel<sup>5</sup> ; while *C. bellensis* Zieten from the Lower Jurassic of Rolzmaden, Germany<sup>6</sup>, has besides a larger diameter a much more crowded system of tunnels.

The genus *Chondrites* ranging from Cambrian to Tertiary does not shed much light on the age of the Grey Shale horizon. But as a *Fodichnia* of Seilacher's *ethological* group of Trace Fossils and falling in his *Nereites facies* indicates, littoral to very shallow water deposition of the grey shales, presence of minute grains of pyrites and marcasite pointing to somewhat oxygen poor surroundings.

**Age :** The more probable age for this horizon appears to be Upper Aptian to Lower Albian<sup>1-3</sup> as indicated by ostracods and foraminifers rather than Hauterivian to Barremian<sup>4</sup>.

**Occurrence :** Grey Shale horizon at Kalkkudi.

**Repository :** The material is deposited in the Department of Geology and Palaeontology of the Maharashtra Association for the Cultivation of Science, Poona-4.

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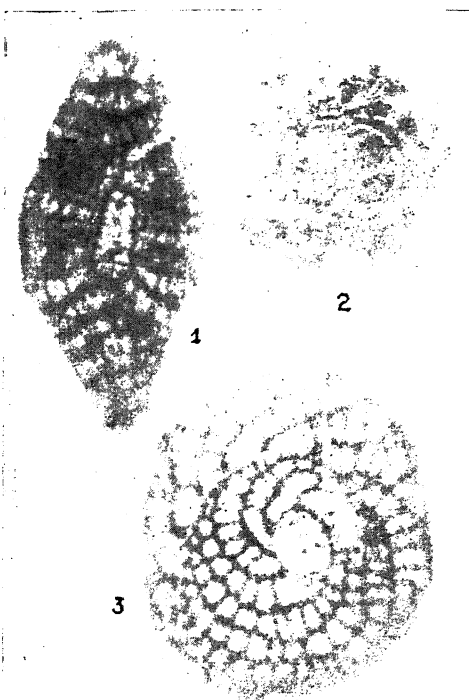
#### NOTE ON THE AQUITANIAN SEDIMENTS OF VINJHAN-MIANI AREA, SOUTHWESTERN KUTCH, GUJARAT

IN Vinjhan-Miani, the fossiliferous limestones and marls belonging to the Lutetian age (Kirthar) are found grading upwards into hard, dark brown coloured marls, which, though lithologically very similar to the underlying Lutetian marls, are easily distinguished from the latter by their different fossil content. The above sequence of beds is exposed about 1 km ENE of Khirasara village.

The overlying marls contain a peculiar type of lamellibranch shells which express affinity with the genus *Pecten*. The characteristic larger foraminifera (*Nummulites*, *Discocyclina*, *Fasciulites*, etc.) as well as its distinctive planktonic foraminifera of the underlying Lutetian do not extend into this horizon.

Thin section studies of the samples of this marl bed have further revealed the presence of *Spiroclypeus ranjanae* Tewari in abundance (Figs. 1, 3) along with rare *Lepidocyclina* (*Lepidocyclina*) sp. (Fig. 2). Typical Oligocene forms like *Nummulites fichteli*, *N. intermedius*, *N. clipeus*, *Lepidocyclina* (*Eulepidocyclina*) sp. and *Miogyopsinoides complanata* (Raju et al., 1970, p. 162) are absent in it. Since the occurrence of *Spiroclypeus ranjanae* has been noted in the Aquitanian beds of Waior in association with *Miogyopsina* S.L., *Miogyopsina* (*Miogyopsinoides*) *dehaarti*, *Lepidocyclina* (*Nephrolepidina*) *sumatrensis*, L. (*Nephrolepidina*) *borneensis*, *Miogyopsina* (*Miogyopsina*) *irregularis*, *Austrotrillina howchini*, etc. (Tewari, 1959), the Aquitanian age for *Spiroclypeus ranjanae* seems favourable. The work of Eames, Banner, Blow and Clarke (1962), who reported this form and *Miogyopsina* together from the Asmari Limestone (Aquitanian), lends support to the Aquitanian age of *Spiroclypeus ranjanae*. In the absence of definite

Oligocene foraminifera, *Spiroclypeus ranjanae* is suggested to be of Aquitanian age; and, as a consequence, the overlying marl horizon with *Spiroclypeus ranjanae* and the lamellibranch shells having affinity with *Pecten* may be dated as Aquitanian.



Figs. 1-3. Figs. 1, 3. *Spiroclypeus ranjanae*, Fig. 1, axial Section; Fig. 3, equatorial section,  $\times 35$ . Fig. 2. *Lepidocyclina* (*Lepidocyclina*) sp., equatorial Section,  $\times 35$ .

The contact between the underlying Lutetians and the overlying Aquitanians is disconformable (see Table I).

TABLE I

|                    |  |
|--------------------|--|
| Aquitanian         | Dark brown, hard, fossiliferous marl bed studded with lamellibranch shell showing affinity with <i>Pecten</i>  |
|                    | ----- Disconformity -----  |
| Lutetian (Kirthar) | Fossiliferous light yellow marl and limestone with <i>Nummulites Fasciolicus</i> , <i>Discocyclina</i> , etc. and Lutetian planktonic foraminifera; unfossiliferous khaki shales and bauxite and conglomerates |

In the light of the aforesaid facts, it can be remarked that:

(a) in Vinjhan-Miani, the Lutetian rocks are directly overlain by the sediments referable to the Aquitanian age;

(b) the presence of Aquitanian directly over the Lutetian suggests that the Oligocene (Nari) is absent in the area under study;

(c) the aforesaid faunal differences observed in the overlying horizon, namely, the presence of lamellibranch shells of *Pecten* affinity, the presence of *Spiroclypeus ranjanae*, and the absence of larger and smaller (planktonic) Lutetian foraminifera, clearly establish this horizon as a separate ecological as well as time rock unit. The fauna in this bed indicates deposition in a very shallow neritic environment, i.e., from the low tide mark down to a depth of 20 metres, where the bivalves are able to survive very suitably. This environment of deposition also makes this horizon distinct from the underlying Lutetians in which the deposition appears to be controlled by the central shelf conditions (40 to 80 m).

The writer expresses his indebtedness to Prof. R. C. Misra. Dr. S. N. Singh, and Dr. K. P. Vimal for their able suggestions and encouragement. He is also indebted to his mentor, Dr. Pratap Singh, for the help in the preparation of this note. This study is being supported by the State Council of Scientific and Industrial Research grant SCSIR/1883/LKO(45)/72.

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#### LOWER AND MIDDLE STONE AGE TOOLS FROM PALGHAT DISTRICT (KERALA)

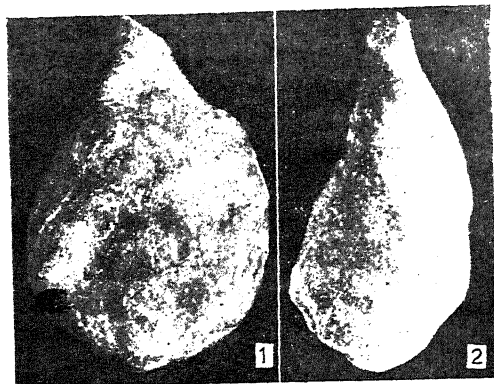
Two Palaeolithic sites have been discovered in Palghat District on the upper region of Kanhirapuzha and Malampuzha rivers.

Collection from Kanhirapuzha river bed comes from the pebble deposit. In this, there are two Hand-axes of the Lower Palaeolithic culture (Figs. 1 and 2) made on gneiss rock. The tools are made on pebbles. Such tools have been reported from Karnataka<sup>1</sup> and they are compared with 'Larsen Type'<sup>2</sup> of South Africa.

Middle Palaeolithic tools (Fig. 3) were obtained from Malampuzha region, which include borers, flakes and fluted cores of quartz. Borers show the character of 'Teri Type' and one among



them is a scraper-cum-borer type. Retouches are seen on the sides and at the end of the borers. On the flake the positive bulb-of-percussion is very prominent.



Figs. 1-2

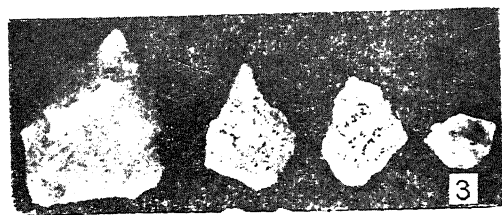


FIG. 3

This is perhaps for the first time that the Stone Age Sites have been discovered in Kerala.

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#### VARIATIONS IN CHEMICAL CONSTITUENTS OF BLOOD PLASMA OF *CLARIAS BATRACHUS* (L.) DURING STARVATION

BILINSKI AND GARDNER<sup>1</sup> showed a more significant increase in the concentration of free fatty acids in blood plasma of rainbow trout in the first two weeks of fasting. In the present study changes in total protein, albumin, globulin, fibrinogen, non-protein nitrogen, iron, calcium, cholesterol, total phosphorus in plasma and glucose in whole blood are recorded at different intervals of starvation.

Fifty fishes of size 20-25 cm were put in a large glass aquarium (90 × 45 × 40) containing tap

water. They were fed four days before starting the experiment. Sampling was done after 1, 2, 5, 10, 15, 30, 45, 60, 75 and 110 days of fasting. On each sampling day five fishes were sacrificed. Throughout the period of experiment, temperature of the aquarium water ranged from 20° C to 35° C. Water of the aquarium was changed every day. There was no mortality of fishes during the period of experiment. Blood was collected after severing the caudal peduncle of the fish. For checking coagulation double oxalate anti-coagulant at the concentration of 4 mg/2 ml of the blood was used. After taking the required amount of the whole blood for the analysis of glucose, it was quickly centrifuged at 3,500 rpm for 15 minutes.

The techniques described by Oser<sup>2</sup> were adopted for the estimation of various chemical constituents.

Total protein, albumin, globulin, fibrinogen, total phosphorus, glucose, iron and calcium showed similar pattern of changes during starvation (Fig. 1).

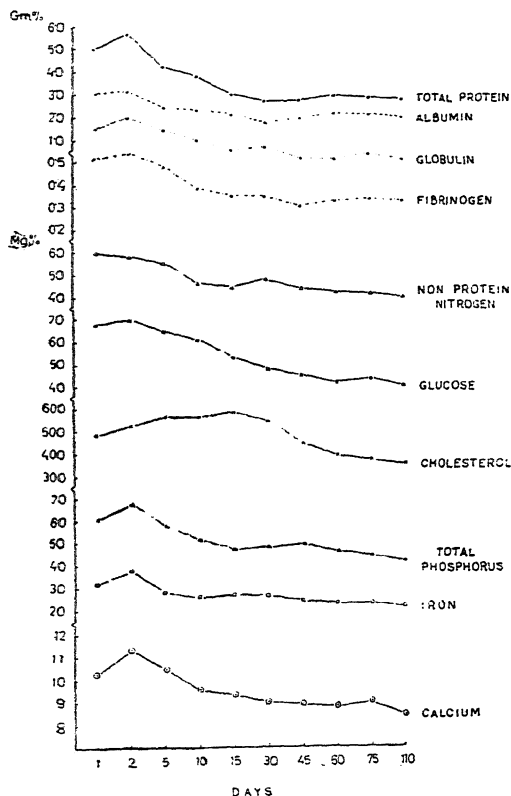


FIG. 1. Variations in chemical constituents in the blood plasma of *C. batrachus* during starvation.

The maximum values were recorded on the second day of starvation, thereafter values decreased gradually. In non-protein nitrogen a gradual fall

was noted. A large increase in the concentration of cholesterol was noted upto 15th day, thereafter the values decreased gradually.

Variations in chemical constituents were due to haemoconcentration and haemodilution. During early days of fasting, the body tissues became more acidic and absorbed water from the blood, and increased the concentration of chemical constituents in the blood<sup>2</sup>. But during later days the mechanism was reversed, where, the release of water from the tissues to the blood resulted in the haemodilution. During starvation stored materials are utilized for basal metabolism and consequently, the concentration of different chemical constituents is diminished.

It can be concluded that during prolonged period of starvation fish acclimatizes itself and its resting metabolism decreases markedly. Initially there is a rapid fall in the concentration of various constituents of the plasma, and as soon as the fish adjusts itself, the decrease becomes gradual and in this way fish endures a prolonged period of starvation.

Grateful acknowledgement is made to Dr. A. Q. Siddiqui for valuable help and advice.

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#### TISSUE SPECIFICITY OF GLYCOGEN SYNTHESIS IN SCORPION *HETEROMETRUS FULVIPES*

STUDIES on the physiology and biochemistry of arachnids have received cursory attention, and there are very few reports on glycogen synthesis in the scorpion<sup>1</sup>. The distribution of biochemical constituents and incorporation of labelled glucose into glycogen in tissues of the South Indian field scorpion, *Heterometrus fulvipes* (Koch), showed tissue specific glycogen metabolic potential in this animal<sup>1,2</sup>. It seems necessary to obtain information about the tissue specificity of glycogen synthesis.

The collection of the scorpions, their maintenance and isotope uptake have already been described<sup>1,2</sup>.

$$\text{Tissue somatic index} = \frac{\text{Tissue wet weight (g)} \times 100}{\text{Total animal wet weight (g)}}$$

Tissue somatic indices of scorpion show variations. They are in the order, Hepatopancreas < Muscle < Ovary (Table I). It is evident that hepato-

pancreas constitutes more than a quarter of the whole body wet weight. This is appreciable since the hepatopancreas, which parallels insect fat body and vertebrate liver, is found to be the important site for biochemical reactions<sup>3</sup>.

In striking contrast to vertebrates, where muscle constitutes about 45% of total body wet weight<sup>4</sup>, the somatic index of muscle of scorpion is only 10.6. From its synthetic ability and carbohydrate content, it has been suggested<sup>1</sup>, that muscle in scorpion does not contribute to the general metabolic pool of the animal. The ovarian somatic index is only 3.7, but the period in which the experiments were performed (February-early March) is the breeding period for *H. fulvipes* which is reflected by its high glycogen content<sup>1,2</sup> and active glycogen synthesis (Table I). However the tissue somatic index has been shown to vary with the season as well as with the physiological state of the animal<sup>5,6</sup>.

TABLE I  
Tissue somatic index, unit activity (counts/min/  
glycogen in gram fresh tissue) and tissue activity  
(counts/min/total glycogen present in whole  
tissue) in various tissues of scorpion

| Tissue         | Number of<br>observations<br>made | Tissue somatic<br>index | Unit activity | Tissue activity |
|----------------|-----------------------------------|-------------------------|---------------|-----------------|
| Hepatopancreas | 7                                 | 27.90                   | 2000          | 2740            |
| Muscle         | 6                                 | 10.61                   | 625           | 326             |
| Ovary          | 6                                 | 3.72                    | 748           | 136             |

Glycogen synthesis in various tissues was also found to vary (Table I). Activity was recorded at 18 hours after the administration of U-C<sup>14</sup> glucose, and this period was found to be optimum for incorporation<sup>1</sup>. When activity was considered for total glycogen present in gram fresh tissue, hepatopancreas is shown to have the highest synthetic ability followed by ovary and muscle. Even when specific activity was considered, the same trend was observed<sup>1</sup>. However, in order to get a true picture of the glycogen synthetic potential of various tissues, the activities have been expressed for total glycogen present in the whole tissue. This expression is known to give a clear picture of the contribution of a tissue to the whole animal metabolism<sup>4</sup>. Data presented indicate that the activity is in the order, Hepatopancreas < Muscle < Ovary. This is interesting since the activity considered for glycogen present in gram fresh tissue showed that the activity of ovary exceeds the activity of muscle. It appears that though the animal is in vitello-

genesis, it does not seem to have a high glycogen synthetic potential as has been observed in insects, which accumulate large amounts of glycogen during vitellogenesis<sup>7</sup>. This high level of carbohydrate content has been shown to facilitate the utilization of dietary proteins in the blow-worm, *Phormia*<sup>8</sup>. The lower synthesis of glycogen in ovary and high synthesis in hepatopancreas indicate a possible mobilization of sugars from hepatopancreas to ovary, since such preferential channeling of energy from visceral tissue to gonadal tissues during reproductive phase is not rare in arthropods<sup>9</sup> and vertebrates<sup>10</sup>.

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three developmental stages: tadpoles without legs, with fore limbs, and with both fore and hind limbs. Adult frogs were also collected from the same area. Liver, muscle, kidney and brain tissues were removed, chopped fine, washed and ground in a mortar and pestle with distilled water in a 1:2 dilution (grams wet tissue per milliliter water). In case of kidney and brain pooled tissue from several tadpoles was used. Hemogenate was centrifuged at 10,000 rpm for 30 minutes and the supernatant so obtained was mixed with 40% sucrose in 1:1 ratio and 0.1 ml applied directly on the separation gel prepared according to Davis<sup>6</sup>. For first 5 minutes 2.5 m Amp. current per tube was applied which was then increased to 5 m Amp. and run carried for 20 minutes at 4° C. After electrophoresis gels were stained for esterase activity<sup>7</sup> and photographs made.

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#### ESTERASE PATTERNS DURING METAMORPHOSIS OF TADPOLE TO ADULT *RANA TIGRINA*

SEVERAL biochemical changes underlie the process of metamorphosis from tadpole to an adult frog. Most studies have demonstrated quantitative changes in enzyme activities during this process<sup>1</sup>. However a notable qualitative variation is found in the tadpole and adult hemoglobins<sup>2</sup>. Recent studies on isozymes have shown altered patterns during development stages indicating a differential gene activity as the basis of development and differentiation<sup>3-5</sup>. In this study using disc electrophoretic technique we report changing esterase patterns during the metamorphosis of tadpole to adult *Rana tigrina*.

Large tadpoles of *R. tigrina* were collected from ponds around Warangal city. These consisted of

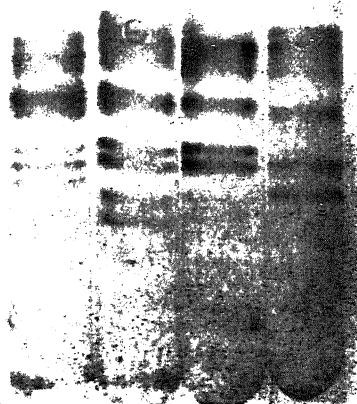


FIG. 1. Disc electrophoretic pattern of esterases of liver tissue from *R. tigrina* tadpole without limbs (I), with fore limbs (II), with both fore and hind limbs (III) and adult frogs (IV).

Figure 1 shows esterase patterns obtained with  $\alpha$ -naphthylacetate as substrate from liver tissue of tadpoles of three developmental stages and the adult frog. The esterase bands from other tissues were faint and could not be identified properly. Where such faint bands could be seen it was evident that the band patterns were different from one another among the above four stages. The liver tissue gave the maximum number of seven to twelve esterase bands which were distinct and

whose identity could be traced from one stage to another. From these patterns it is evident that majority of the esterase bands are common in all stages. This identity is less apparent because of the extremely low intensity of some bands in some stages. However, in some stages there is an absence of certain bands. The absence of a band need not necessarily mean that such isozyme is not produced at all although such possibility cannot be ruled out. These zymograms then indicate that some esterase isozymes either are not produced in a particular stage of development or are produced in extremely low quantities. This study however shows that esterase isozymes vary from one stage of development to another and this might have a genetic basis in the activation of specific genes at particular stages as has been shown in other cases<sup>3</sup>. Such difference in esterase patterns were also observed in an electrophoretic study of *R. cates-biana*<sup>5</sup>. Our study confirms this observation and suggests the differential gene activity as the basis of development and differentiation.

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#### ON THE MALFORMED CAUDAL NEURO-SECRETORY SYSTEM IN *CLARIAS* *BATRACHUS* L.

DURING the course of a comparative anatomical study on the caudal neurosecretory system in teleosts<sup>1</sup>, eleven specimens of *Clarias batrachus* were found to have aberrant tail. Although their caudal fin was normal in appearance, the caudal portion was tapering. This has promoted us to study the histomorphology of the caudal neurosecretory system in these specimens.

After fixing the caudal portion of the fish in Bouin's fluid for 24 hours and washing with 70% ethanol, the sample was dissected under a low power microscope. It is observed that the preterminal vertebra was irregular, appear knobby in shape and

is invaded by irregularly modified tissue of the spinal cord. The integument in the caudal extremity is closely connected with fibrous tissue. The spinal cord is distended dorsoventrally and is narrowed down gradually towards the caudal end.

A section of the spinal cord, prepared after histological technique<sup>2</sup>, shows wide central canal and dislocated urophysis (Fig. 1). The central canal becomes irregularly widened and contains hyaline cerebrospinal fluid. There is Reissner's fibre coiled up in the form of knots. The central canal is lined up with thick layer of ependymal cells from which processes emerge and end on the periphery of the spinal cord. The Dahlgren cells are concentrated in the attenuated portion of the caudal spinal cord. They are distributed dorsal to the urophysis. A few Dahlgren cells, which line a small isolated canal, are also seen at the extreme end of the spinal cord. The other elements present in this region are glia and ependymal cells.

The urophysis is a small, irregularly shaped region showing mostly medullary fibres and numerous capillaries. There are large Herring bodies as well as



FIGS. 1-2. Fig. 1. Sagittal section of the caudal spinal cord showing wide central canal (CC) and malformed urophysis (arrow). Dark outline around the central canal shows distribution of ependymal cells. Haemalum-eosine,  $\times 17$ . Fig. 2. Enlarged portion of the malformed urophysis showing several medullary fibres and Herring bodies (arrows) of various size. Haemalum-eosine,  $\times 270$ .

small sized droplets of the neurosecretory material (Fig. 2).

In normal specimens of *C. baruchas*, the caudal neurosecretory system has a narrow central canal; the enlarged ventral portion of the spinal cord called trophysis and a filum terminale. The Dohlgren cells are dorsally and are numerous in the region where the trophysis begins to bulge out.

The malformed caudal neurosecretory system is recorded only in *Anacilla japonica*. The occurrence of Dohlgren cells in a limited area of the caudal spinal cord and dislocated trophysis in *C. baruchas* is in agreement with the finding of Imai<sup>4</sup>, who regards them as products of regeneration. It is possible that the malformed nature of this system in *C. baruchas* is due to the regenerated portion of the tail which met with an accident in the nature. If it is so, the enlarged portion of the central canal would form an important route for discharge of secretory products in it and thus would serve as an important link in the functional efficiency of the system.

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# NEW RECORD OF THE ROTIFER *TRIPLEUCHLANIS PLICATA* (LEVANDER) FROM INDIA

IN India, Eucharid rotifers are little known. Stewart<sup>1</sup> reported one species from Tibet, Edmondson and Hutchinson<sup>2</sup> six species from Punjab and Kashmir, Nayar and Nair<sup>3</sup> one species from Kerala and Dhanapathi<sup>4</sup> eight species including a new species from Andhra Pradesh. During limnological investigations three specimens of the rotifer *Tripleuchlanis plicata* were obtained from Municipal tank, Kalkalur (Krishna Dt., A.P.) during January 1974. The locality of collection was with dense growth of *Hydrilla*, diatom species of *Asterionella*, *Fragilaria*, *Gyrosigma*, and *Narvicula*, and green alga *Closterium*. The physico-chemical conditions of water at the time of collection were temperature 20°C, pH 7.4, dissolved oxygen 3.4 ml/L, carbonates 30 ppm, bi-carbonates 140 ppm. It is being reported for the first time from India.

**Description.**—*T. plicata* is ovoid. Dorsal plate of the lorica with emargination posteriorly and the ventral of same size as dorsal. Lateral longitudinal sulci are separated by cuticular flange giving bellows

effect laterally. Mastax malleate type, with overlapping teeth in each uncus. Foot glands long including a pair of accessories. Foot three jointed, projects beyond the lorica; first foot joint covered by small cuticular plate. Toes short and parallel (Fig. 1). Measurements: length of the body 165 µ, of dorsal plate 84 µ, of ventral plate 98 µ, and of toes 28 µ.

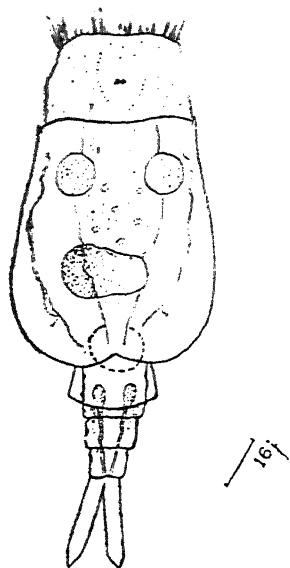


FIG. 1. *T. plicata*, dorsal view.

**Discussion.**—Myers<sup>5</sup> stated that "*T. plicata* is common in the salt water of bays and inlets, wherever there is growth of marine algae; it is the only marine Eucharid rotifer known". Rodewald<sup>7</sup> described *T. plicata* var. *razelmi* from brackish waters of Rumania, with long and narrow lorica, deep rounded cut for dorsal plate and longer cuticular plate on the foot than *T. plicata*. Anne Thane-Fenchel<sup>8</sup> reported it from marine waters of Denmark. Dr. P. J. Donner, Austria, stated (personal communication) that he has obtained it from a freshwater reservoir in Madras.

It was not found in my earlier collections from several districts in Andhra Pradesh, Ootacamund (Tamil Nadu), and Ranchi (Bihar). I consider occurrence of *T. plicata* in freshwater as very rare but, more detailed study is required to know about its distribution.

Thanks are due to Dr. P. J. Donner (Austria) for his suggestions. Thanks are also due to Sri. S. Rama Rao, Head of the Department of Zoology and Sri. P. Srirama Murty, Senior Lecturer in Chemistry, D.N.R. College, Bhimavaram, for

providing the necessary facilities to carry out the work.

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#### NATURAL OCCURRENCE OF BACTERIAL LEAF STREAK DISEASE OF RICE ON *ORYZA PERENNIS*

BACTERIAL leaf streak disease of rice (*Oryza sativa*), caused by *Xanthomonas translucens* f. sp. *oryzicola* (Fang et al.), Bradbury, is widely distributed in tropical Asia. Under favourable weather conditions, the disease spreads in a severe form and causes as much damage as bacterial leaf blight<sup>1</sup>. The role of seed transmission in the perpetuation of the disease has been shown<sup>2</sup>, however, the part played by the weeds in transmitting the disease is little understood. The stagnant water in marshy ponds and streams located in the vicinity of rice fields support the growth of a perennial wild rice, *Oryza perennis*, which is distributed widely in Uttar Pradesh, Madhya Pradesh, Bihar, West Bengal, Orissa, and Andhra Pradesh<sup>1</sup> which form the major part of the rice belt in India. The role of *O. perennis* as an alternative host in the epidemiology of this disease is investigated.

In the beginning of the wet season during the year 1973 before the paddy crop was transplanted in the fields, it was observed that the leaves of *O. perennis* exhibited severe symptoms of streak disease similar to that on rice plants under natural conditions in and around Cuttack, Orissa State. In course of time, the transplanted paddy crop nearby was also severely diseased.

Diseased leaf tissues of *O. perennis* when placed in water exhibited typical bacterial oozing from the cut ends. The bacterium was isolated on potato-sucrose-agar medium and identified as *Xanthomonas*. Fifteen different wild rice species including the natural host of this isolate, *O. perennis* and one month old rice (*O. sativa*) cultivar, T(N)1, raised in pots were artificially inoculated by swabbing the bacterial suspension with muslin cloth obtained

from 48 hr old culture on potato-sucrose-agar medium<sup>3</sup>. Inoculated plants were kept in the open at  $30 \pm 2^\circ\text{C}$ .

Typical water soaked, translucent, interveinal streaks appeared on the leaves of *O. perennis* and the rice cultivar, T(N)1, four days after inoculation and subsequently in a few days they turned reddish brown. The bacterium re-isolated from the infected leaves of cultivar, T(N)1, brought about typical disease symptoms on *O. perennis*, four days after inoculation. Six of the wild species, i.e., *O. barthii*, *O. latifolia*, *O. officinalis*, *O. malampuzhensis*, *O. glaberrima* exhibited susceptible reaction, while *O. grandiglumis*, *O. punctata*, *O. alta* and *O. australiensis* showed resistant reaction. However *O. eichingeri*, *O. granulata*, *O. ridleyi*, and *O. brachyantha* did not exhibit any symptom.

Seeds harvested from naturally infected field with bacterial leaf streak<sup>2</sup> and from artificially inoculated plants<sup>7</sup> were found to carry the inoculum from one season to the other. A few wild rice species including *O. perennis* were found to be susceptible to this disease under artificial inoculation tests at the International Rice Research Institute, the Philippines<sup>3,4</sup>. The natural occurrence of this disease on *O. perennis* and the ease with which it passes to cultivated rice and from rice back to the wild rice suggests that *O. perennis* may act as an alternative host and play a role in the epidemiology of the disease.

Deep appreciation is extended to Dr. S. Y. Padmanabhan, Director and Dr. N. K. Chakrabarti, Head, Division of Plant Pathology, for their encouragement and for providing facilities. Thanks are also due to Mr. P. J. Jachuck for supplying the wild rices and to Dr. R. Sridhar for critically going through the manuscript.

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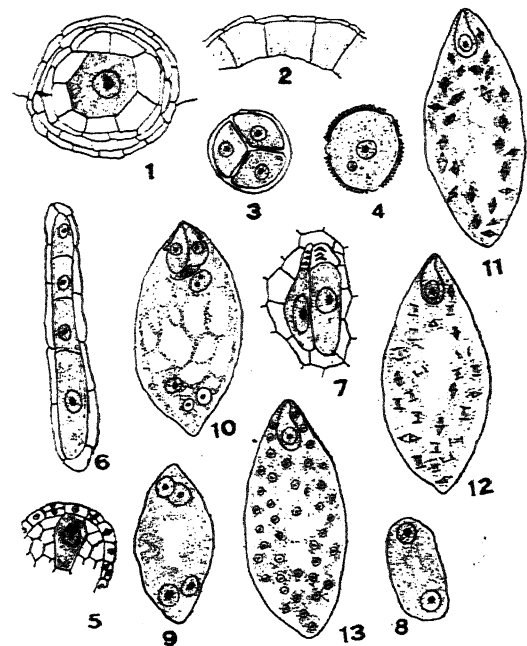
# EMBRYOLOGICAL STUDIES IN *RICHARDIA SCABRA* LINN.

RECENT contributions to the embryology of the family include those of Fagerlind (1937), Shivaramaiah and Sundara Rajan (1973).

The material for the present work was collected round about Bangalore. The flower buds were fixed in F.A.A. and the usual embryological procedure was followed.

*Richardia scabra*, Linn. belongs to the tribe Spermacoceae.

A transection of an young anther lobe shows an epidermis, an endothecium, a middle layer, a tapetum and one microspore mother cell (Fig. 1). The tapetal cells are uninucleate throughout and are of glandular type. Endothecium exhibits fibrillar thickenings at the time of anther dehiscence (Fig. 2). Microspore mother cells undergo reduction division to form tetrahedral tetrads of microspores (Fig. 3). Mature pollen grain has a thick exine with glandular projections and a thin intine. It is shed at the 2 celled stage (Fig. 4).



FIGS. 1-13. Fig. 1. T.s. of anther lobe.  $\times 300$ . Fig. 2. Fibrillar endothecium,  $\times 300$ . Fig. 3. Microspore tetrad,  $\times 700$ . Fig. 4. 2-celled pollen grain,  $\times 300$ . Fig. 5. Archesporial cell. Figs. 6 and 7. Tetrads of megaspores.  $\times 300$ . Figs. 8-10. Development of embryosac.  $\times 300$ . Figs. 11-13. Free nuclear endosperm and zygote.  $\times 300$ .

The ovary is inferior with pendulous ovules. The nucellus is highly rudimentary. At the

archesporial cell stage it is represented by a single cell. Even this degenerates at the megaspore mother cell stage. The nucellus is of the *Hautontia* type (Fagerlind, 1937). A single hypodermal archesporial cell is differentiated and it directly functions as the megaspore mother cell (Fig. 5). The megaspore mother cell undergoes reduction division to give rise to generally a linear tetrad of megaspores. Occasionally juxtaposed twin tetrads are also noticed (Figs. 6 and 7). Of the four megaspores the chalazal alone is functional. The functional megaspore gives rise to an 8 nucleate embryosac of the *Polygonum* type (Figs. 8-10). In spite of the presence of twin tetrads no twin embryosacs have been observed. The organised embryosac is 7 nucleate with an egg apparatus having beaked synergid and a median egg.

Fertilisation is porogamous. The endosperm development is free nuclear (Figs. 11-13). The zygote remains quiescent for a long time until about 200 endosperm nuclei are formed.

Our thanks are due to the Principal and Head of the Department of Botany, St. Joseph's College, Bangalore, for facilities.

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## ON FOLIAR TERMINAL VESICULOSE SCLEREIDS IN *GOUPIA GLABRA* AUBL.

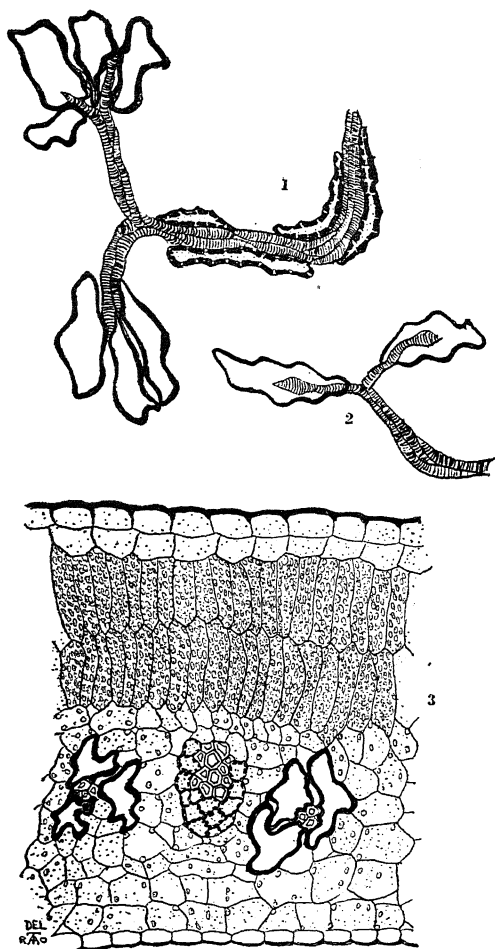
IN the course of a thorough systematic reinvestigation on the several foliar sclereid bearing taxa mentioned in the works of Solereder<sup>1</sup>, and Metcalfe and Chalk<sup>2</sup> the writers came across an interesting occurrence of terminal vesiculose sclereids previously not reported in the leaf expanses of *Goupia glabra*, an aberrant taxon from Amazon, British Guiana and Columbia. This feature is studied in detail in view of the progressive systematic status of this taxon into a separate family, Goupiaceae.

### MATERIALS

*Goupia glabra* Aubl. South America, Amazon, Basin of Rio Madera, B. A. Krukoff 5943 (LE); Amazon, Juijirimom Bandel, Richard Evans Schultes et Isidoro Cabrera 149504 (LE); British Guiana, Near Bartica, N.Y. Sandwith 379 (LE).

A perusal of the literature has revealed that but for a succinct statement that 'mesophyll composed of two layers of palisade tissue, and a broader spongy region, containing branched sclerenchymatous elements' (Metcalfe and Chalk)<sup>2</sup> there is no detail

information available on the types of sclereids encountered in the leaf expanses of this taxon. It is evident from the above statement in agreement with Foster<sup>3</sup> that earlier workers have not employed the clearing technique with advantage to know the overall pattern of sclereid distribution and the relationship with vein-endings.



FIGS. 1-3. Fig. 1. Surface view of vesiculose sclereids at the vein-endings and also vermiform sclereids closely adpressed to the minor veins,  $\times 60$ . Fig. 2. Veinlet-ending with a single vesiculose sclereid,  $\times 60$ . Fig. 3. Transection of the leaf showing vesiculose sclereids in the spongy tissue,  $\times 60$ .

The interesting feature of the present study of cleared leaves, transverse and paradermal sections is the discovery of terminal vesiculose sclereids at the vein-endings. Its terminal disposition is relatively constant and this pattern seems to be constant in almost all the cleared leaves. The sclereids

conform to vesiculose type *sensu* Rao and Bhupal<sup>4</sup>. In cletred lamina they are parallelly oriented to the surface of the lamina and possess birefringent thin walls with wide lumina without any contents. This type varies from sub-spherical to irregularly lobed form, but invariably sac-like at the vein-endings (Figs. 1, 2). Frequently, they appear in groups of 2 to 4 in close juxtaposition. Infrequently, single large sac-like sclereid is found at the vein-ends, almost enclosing the terminal portion of the veinlet (Fig. 2). In transections they are disposed in the broad spongy region in groups of two to three (Fig. 3). In size they are larger than the surrounding spongy cells and irregularly oriented around vascular bundles. Further, it is evident from an analysis of venation pattern of the cleared leaves that the midrib and the other major veins possess compact sclerenchymatous sheaths, whereas the minor veins have less sclerenchyma but only a few scattered isolated sclereids with round wall endings (Fig. 1) apparently resembling the vermiform type of sclereid. These sclereids are distinct from the vesiculose sclereids in their specific topographical disposition and orientation along the veins. They have thick cell walls with conspicuous pits and narrow lumen. Infrequently, they are found intruding into the mesophyll.

This taxon is incorporated in Celastraceae in Bentham and Hooker System. Losner<sup>5</sup> erected for this aberrant taxon a sub-family Goupioideae within the Celastraceae. Miers<sup>6</sup> recommended raising this taxon to distinct family level, Goupiaaceae. According to Dr. T. A. Spraguel (quoted by Metcalfe and Chalk)<sup>1</sup> this taxon has sufficient distinct characters to exclude from the Celastraceae. Recent researches have shown undoubtedly that the occurrence of foliar sclereids in many phanerogams are of obvious positive taxonomic significance. The present finding of terminal vesiculose sclereids at the vein-endings will certainly be a helpful diagnostic character with other distinct characters mentioned before<sup>1</sup> to accommodate this genus into a family in its own right.

The senior author is grateful to the authorities of the Academy of Sciences of U.S.S.R., the Department of Science and Technology and the Director of Botanical Survey of India for providing him the opportunity of visiting U.S.S.R. under Indo-Soviet Scientific and Cultural Exchange programme during 1973-74. Furthermore, he is grateful to the authorities of V. L. Kumarov, Botanical Institute, Leningrad, for the gift of leaf-specimens of several sclereid bearing taxa of flowering plants.

Botanical Survey of India, T. ANANDA RAO.  
76, Lower Circular Road, MRS. J. BHATTACHARYA.  
Calcutta-14, July 10, 1974.



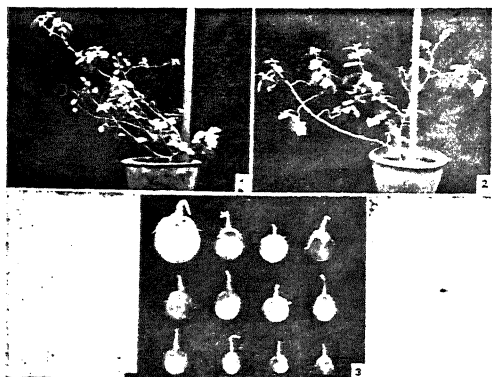
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### ON THE OCCURRENCE OF NATURAL HYBRIDIZATION BETWEEN *SOLANUM INCANUM* LINN. AND *SOLANUM MELONGENA* LINN.

EXPERIMENTAL hybrids between *Solanum incanum* and *Solanum melongena* have been well established, Bhaduri (1951)<sup>1</sup>; Zutshi (1968)<sup>2</sup>.

While on a collection trip to the Cheruma tribe in search of primitive cultivars of economic plants of Kerala an extremely spinous form of *Solanum incanum* with small green fruits was found growing on the roadside near Shoranur.

Fruits of this plant were collected and seeds sown in the Ethnobotanical Garden of the Field Research Laboratory, University of Madras. Amongst the seedlings raised which were similar to the plant collected (Fig. 1), one plant stood out by its vigorous growth, large pubescent leaves and rudimentary spines. This aberrant seedling had larger flowers, and fruits which were purple in colour, a character not so far seen in *Solanum incanum* (Fig. 2).



FIGS. 1-3. Fig. 1. A normal *Solanum incanum*, Linn. plant. Fig. 2. The aberrant plant. Fig. 3. Segregation for fruit size and colour from the aberrant plant.

Selfed seedlings of this aberrant plant showed segregation for colour and size of fruits—the largest

fruit resembling a round *Solanum melongena* and the smallest similar to *Solanum incanum* parent. The different types of fruits present in the segregating generation are illustrated in Fig. 3. We can thus infer that hybridization between *Solanum incanum* and *Solanum melongena* occurs in nature.

Some of the forms of the segregates correspond to the description by Hooker (1885)<sup>3</sup> for *Solanum coagulans*. The hybrid nature of *Solanum coagulans* and the possibility of its being a primitive cultivar of *Solanum melongena* is thus indicated by this study.

I wish to thank Dr. E. K. Janaki Ammal for her guidance in this study, which is part of the project "Ethnobotanical studies of South Indian aboriginal tribes" financed by the Indian National Science Academy.

Centre for Advanced

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### THE NATURE OF PECTIC ENZYMES PRODUCED BY *PHYTOPHTHORA PARASITICA* VAR. *SESAMI* IN TWO CULTURE MEDIA

*Phytophthora* blight is causing serious damage to sesamum crop in Rajasthan (Prasad *et al.*, 1966). The disease has earlier been reported from Gujarat (Kale and Prasad, 1957). In Rajasthan the disease was severe in 1973 around Udaipur. Isolations from diseased tissues yielded pure cultures of the pathogen. This is a preliminary report on work in progress in our laboratory on the host-parasite relations of this disease.

The pathogen was grown on potato pectin medium (Talboys and Busch, 1970) and Asthana and Hawker's medium (Dube, 1971) with pectin as the carbon source. Culture filtrates were assayed for pectic enzyme production by the pathogen after seven days. The identification of hydrolytic or *trans*-eliminative cleavage of the pectic substances was made spectrophotometrically by Thiobarbituric acid test of Neukom (1960), as modified by Sherwood (1966). Spekolsingle beam spectrophotometer was used for the purpose of determining the absorption spectrum over the range of 480-580 nm. Occurrence of a

peak at 510 nm indicated hydrolytic cleavage by a polygalacturonase and a peak at 547-550 nm was evidence for a *trans*-eliminative split and therefore for pectin lyase.

Seven days old culture filtrate of the fungus growing on potato-pectin medium when assayed showed maximum absorption at 510 nm (Fig. 1). This peak at 510 nm occurred at a pH range of 5 to 8; there was no activity below pH 5 and above 8. The pH for maximum enzyme activity was found to be 6. There were peaks showing enzyme activity on both sodium polypectate and pectin with similar effect of pH on the reaction mixture. There was no peak at any pH on 547 or 550 nm indicating absence of pectin-lyase.

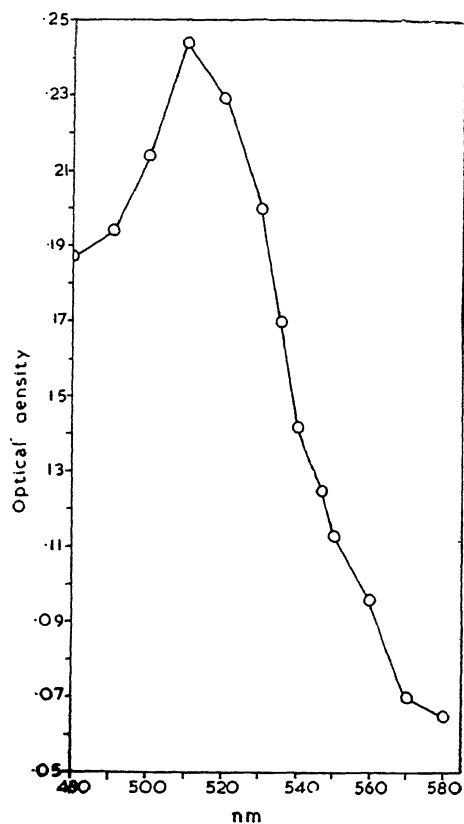


FIG. 1. Absorption spectrum showing hydrolytic cleavage of NaPP at pH 6.

On Asthana and Hawker's medium, the culture filtrate, after seven days of fungal growth, showed the presence of a pectin lyase (Fig. 2). Distinct peak at 550 nm occurred at pH ranging between 5 to 8, though at pH 5 the peak was highest.

This evidenced the presence of a pectin lyase. Here too, the enzyme activity occurred on both the substrates, viz., sodium polypectate and pectin though the pH for maximum enzyme activity did vary in the two cases. On pectin the highest peak was recorded at pH 6.

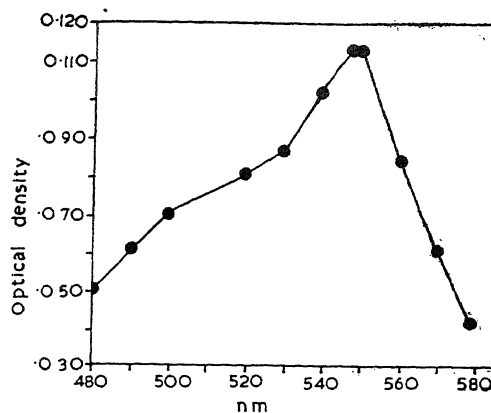


FIG. 2. Shows *trans*-eliminative cleavage of pectin at pH 5.

The results are interesting on two scores: (i) the culture conditions, viz., the nature of growth medium, determined the nature of the enzyme production by the pathogen and, (ii) the pH for maximum activity was in the acidic range, for the pectin lyases are usually reported to be active in the alkaline range.

Grateful thanks are due to Professor Y. D. Tiagi for providing laboratory facilities and for critically reading the manuscript.

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# MORPHOGENESIS IN EMBRYONAL CALLUS OF *EUPHORBIA PULCHERRIMA* IN VITRO

REPORTS on the *in vitro* morphogenesis of embryo and the induction of polyembryony are available<sup>1,2</sup>. In *Euphorbia pulcherrima* the induction of the hypocotyledonary buds in seed cultures was earlier described<sup>3</sup>. This communication deals with the embryo proliferation and its subsequent differentiation into embryoids and plantlets.

Mature seeds of *Euphorbia pulcherrima*, an ornamental plant, were collected and the seed coat removed. Following the aseptic technique, the decoated seeds were cultured on a modified Whites' medium without IAA<sup>4</sup>, containing 2% sucrose (BM) and also on BM with supplements like coconut milk (CM), IAA, GA<sub>3</sub>, kinetin and casein hydrolysate (CH) either individually or in different combinations. The cultures were maintained under controlled conditions of temperature, light and humidity.

On BM and BM + CM (10%), the seeds showed upto 90% germination but callusing was not noted. But on all the other nutrient media tried callus formation was observed and the percentage of cultures showing seed germination, differentiation of hypocotyledonary buds and callus are summarised in Table I. On BM + kinetin (1 ppm) only 5% and

TABLE I

Percentage of cultures showing seed germination, shoot buds and callus

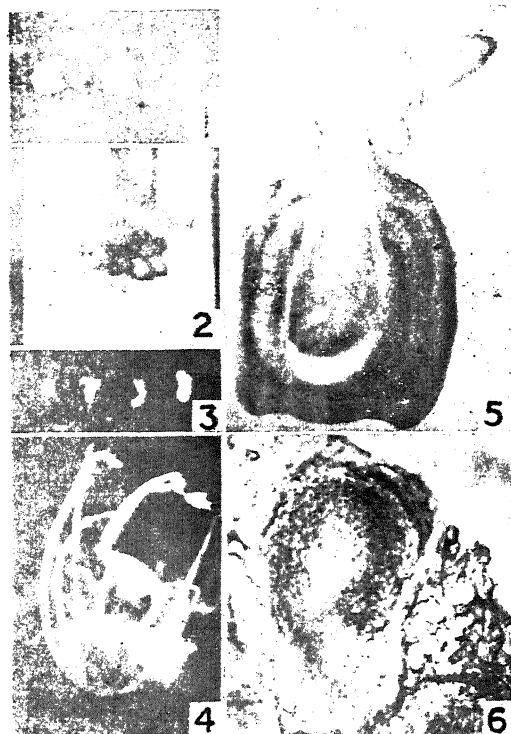
| Nutrient media                             | Germination | Shoot Buds | Callus |
|--|-------------|------------|--------|
| BM   | 90          | 35         | ..     |
| BM + CM (10%)                              | 90          | 35         | ..     |
| BM + IAA (1 ppm)                           | 70          | 35         | 10     |
| BM + IAA (1 ppm)<br>+ CM (10%)             | 50          | 35         | 10     |
| BM + kinetin (1 ppm)                       | 70          | 20         | 5      |
| BM + kinetin (1 ppm)<br>+ CM (10%)         | 35          | 25         | 15     |
| BM + GA <sub>3</sub> (1 ppm)               | 70          | ..         | ..     |
| BM + GA <sub>3</sub> (1 ppm)<br>+ CM (10%) | 65          | 10         | 10     |
| BM + 2, 4-D (1 ppm)                        | ..          | ..         | ..     |
| BM + 2, 4-D (1 ppm)<br>+ CM (10%)          | 25          | 8          | 35     |

Growth period : 10 weeks. Average of 24 cultures.

on BM + kinetin (5 ppm) + CH (500 ppm); BM + kinetin (5 ppm) + 2, 4-D (2 ppm); BM + kinetin (5 ppm) + 2, 4-D (2 ppm) + CH (500 ppm); BM + kinetin (1 ppm) + IAA (1 ppm) + CH (500 ppm) and BM + 2, 4-D (2 ppm) + CH (500 ppm) all the cultures produced callus.

The seeds within 3 days of culture enlarged rapidly followed by the emergence of radicle. The latter swelled considerably and the hypocotyl region proliferated (Fig. 1) profusely yielding a mass of yellowish-brown, fleshy callus after 2 weeks of culture. In a few cultures that the callusing was so profuse the entire seed lost its identity within the fast growing callus.

Interestingly, in about 6 weeks numerous embryoids invariably differentiated in the callus (Figs. 2, 3). These embryoids were pale-yellow and developed into plantlets (Fig. 4). The development of these embryoids was not synchronous and several embryoids at different stages of their ontogeny were seen intimately mixed with the mature ones. The callus was subculturable and continued to differentiate embryoids.



FIGS. 1-6. Fig. 1. Seeds grown on BM + CH (500 ppm) + 2, 4-D (2 ppm) showing the formation of callus. Fig. 2. A 6-week-old culture on BM + kinetin (5 ppm) + CH (500 ppm) with a mass of callus and proembryoids. Figs. 3, 4. Embryoids and plantlets. Fig. 5. L.s. of a seed after a week in culture to show the origin of callus; note the proliferated hypocotyl. Fig. 6. A portion of callus in section passing through a proembryoid.

Histological preparations of the callusing seeds revealed that the cotyledons and the shoot apex remained unaffected and the callus originated from

the entire hypocotyl region (Fig. 5). The actively growing callus contained parenchymatous cells of diverse sizes and shape. The cells were largely uninucleate, densely cytoplasmic and were studded with starch grains. Occasionally binucleate cells were also noted.

The embryoids originating from the callus elongated considerably along the radicle-plumule axis, but the differentiation of cotyledons was very poor. The radicle penetrated into the medium and organized into a primary root with a prominent root cap. Anatomically the callus comprised several proembryoids (Fig. 6) which were easily recognised by their typical organization. In several cultures the endosperm also exhibited rapid proliferation yielding a mass of tissue capable of differentiation. Further studies on endosperm morphogenesis are in progress.

I am indebted to Professor M. S. Chennaveeraiah for his help and encouragement.

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#### **SCHIZOTETRANYCHUS ANDROPOGONI (HIRST) (ACARINA: TETRANYCHIDAE) A NEW RECORD OF PADDY PEST IN INDIA**

PADDY has so far been reported to be attacked in India only by one species of mite, i.e., *Oligonychus oryzae* (Hirst<sup>1</sup>). Recently, a thorough survey of paddy crop at Chinsurah, Kalyani and Krishnanagar areas of West Bengal revealed the existence of another mite, *Schizotetranychus andropogoni* (Hirst) which infested several varieties of paddy in pest form and did considerable damage to crop during August–September 1972. Earlier, this mite has been reported as pest on sugarcane (*Saccharum officinarum*<sup>2-3</sup>), on *Dicanthium annulatum*<sup>4-5</sup> and *Saccharum spontaneum*<sup>6-7</sup>.

This mite was in small colonies mostly on the under-surface of leaves but when the infestation was severe, its occurrence on upper surface of leaves was also quite common. These mites cause whitish patches in rows arranged irregularly on either sides of midrib. All the patches remain covered with thin webs where the mite colonies lived. Paddy fields having serious injuries of mites looked sickly and turned yellowish.

Sixteen varieties of paddy were examined in the field for their relative susceptibility to the attack of *S. andropogoni*. Population of mite was highest on NC 918 (460) and lowest on Satika (20). IR 8 was completely free from mite attack. The population of mite was heavy during August–September, gradually dwindled during October and became nil during end of November.

TABLE I

| Sl. No. | Name of variety          | Population per 10 leaves |
|---------|--------------------------|--------------------------|
| 1       | NC 918                   | 460                      |
| 2       | CH <sub>4</sub>          | 390                      |
| 3       | Ratna                    | 198                      |
| 4       | Krishna                  | 166                      |
| 5       | CR <sub>2</sub> /10-1114 | 138                      |
| 6       | Vijaya                   | 135                      |
| 7       | Padma                    | 120                      |
| 8       | Latisail                 | 116                      |
| 9       | IR 22                    | 109                      |
| 10      | OC 52                    | 105                      |
| 11      | IET 849                  | 101                      |
| 12      | Dhasamanik               | 61                       |
| 13      | IR 20                    | 55                       |
| 14      | Kanchi                   | 54                       |
| 15      | Satika                   | 20                       |
| 16      | IR 8                     | 0                        |

Two species of *Oligonychus*, viz., *O. indicus* (Hirst) and *Oligonychus* sp. were associated but their population were insignificant. Two species of predatory phytoseiid mites, viz., *Amblyseius fallacis* (Garman) and *A. longispinosus* (Evans) and one species of Tydeidae, viz., *Pronematus* sp. were abundantly found on all the varieties of paddy and found very efficiently feeding on the eggs of *S. andropogoni*. One species of Acaridae were also found to occur on paddy but its role could not be ascertained.

The author is grateful to Dr. A. P. Kapur, Director, for the facilities. Thanks are also due to the authorities of the Agricultural farms for the permission to record observations.

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## SHORT SCIENTIFIC NOTES

**New Records of Alternate Hosts of Tobacco Caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera. and Bihar Hairy Caterpillar, *Diacrisia obliqua* Wlk.) (Arctiidae: Lepidoptera)\***

Tobacco caterpillar, a polyphagous pest which had been reported to attack about 103 host plants<sup>1,2</sup>, was found damaging gladiolus by feeding on the flowers extensively at the experimental farm, Hessaraghatta, of the Indian Institute of Horticultural Research. A spray of 0.05% paration emulsion could effectively control the pest.

Bihar hairy caterpillar, a similiar polyphagous pest with over 40 host plants<sup>3,4</sup>, caused severe damage to the globe artichoke (*Cyanara scolymus*) by feeding on the leaves. These alternate hosts of both the pests are placed on record for the first time.

Thanks are due to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, Bangalore, for encouragement.

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**First Record of a Host for the Chalcid Parasite *Brachymeria croceogastralis* Joseph *et al.***

During the course of rearing of *Perina nuda* Fabr. (Lepidoptera : Lymantriidae) which fed on the leaves of fig trees in our college orchard in October 1960, a number of chalcids emerged from the pupae in the laboratory. The parasites were identified as *Brachymeria croceogastralis* Joseph *et al.* (Chalcididae). Joseph *et al.*<sup>2</sup> have described the species from specimens collected at Bangalore (India) from unidentified pupa but no specific host of the parasite has been reported. The parasite complex mentioned by Cherian and

Israel<sup>1</sup> does not include *B. croceogastralis* and as such forms a first record.

Thanks are due to Prof. K. J. Joseph for having identified the parasite and to Prof. T. R. Subramaniam for the facilities provided.

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**Leaf Margin Roll Gall on *Ficus tomentosa* Roxb. Caused by a Psyllid *Mycopsylla gardenensis* Bhanotor (Hemiptera)**

The psyllid, *Mycopsylla gardenensis* Bhanotor has been observed for the first time to cause leaf margin roll gall on *Ficus tomentosa* Roxb. in and around Madurai region. Feeding by the psyllids results in a typical phytotoxemia of primary tissue malformation on the leaves. The apex of the leaf is rolled and folded back on the underside upto half way of the leaf blade and/or leaf margins on the lateral sides are sharply folded and fixed on the lower side. The entire blade becomes rolled, crinkled and spirally twisted into a tubular structure in young leaves. The rolling of the blade is almost accompanied by swelling, crinkling and curling.

Groups of greenish flattened nymphs remain feeding inside the leaf rolls in a cottony cushion. Whitish waxen threads project from the extremity of abdomen giving an appearance of masses of cotton to the nymphal aggregation. Adults disperse throughout the branches. Honey dew, secreted copiously by the nymphs, drops downwards and attracts several flies and wasps.

A pit gall caused by *Trioza* sp. on *Ficus religiosa* L. and a pouch gall on *Ficus glomerata* Roxb. by *Pauropsylla depressa* Crawford, have been reported from India<sup>2</sup>. Trotter<sup>3</sup> has observed a leaf margin roll gall on *Ficus nervosa* Heyne<sup>1</sup>, caused by an unknown psyllid from Formosa. This is the first record of leaf margin roll gall on *Ficus tomentosa* Roxb. caused by a psyllid.

The authors are thankful to Dr. D. Hollis, British Museum (Natural History), London, for having kindly identified the psyllids.

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#### *Achlya stellata* de Bary—A New Record from India

*Achlya stellata* has been recently isolated from a water sample from Gorakhpur which forms the first record of this fungus from India. The following description of the isolate is made from bacteria-free cultures growing on sterilized hemp-seed halves in sterile distilled water at 22–25° C.

#### *Achlya stellata* de Bary

*Mycelium* moderately extensive, diffuse; two-week-old colony 1.5–2.5 cm in diameter; principal hyphae slender, branched, 70–105  $\mu$  in diameter at base. *Zoosporangia* moderately abundant fusiform or clavate, less frequently naviculate or filiform; 275–550  $\mu$  long by 18–45  $\mu$  in diameter, predominantly 350–500  $\times$  25–35  $\mu$ ; renewed sympodially, occasionally cymosely; zoospore discharge achlyoid; spore cluster not persistent; encysted spores 7.3–10  $\mu$  in diameter. *Gemmae* absent. *Oogonia* abundant, terminal on main hyphae or on lateral branches which are commonly curved or rarely once coiled; oogonia spherical pyriform, often with a neck, the basal septum often having an inward projection; 35–84  $\mu$  in diameter predominantly 55.5–70  $\mu$  inclusive of ornamentations. Oogonial wall unpitted; densely ornamented with conical or mammiform papillae. *Antheridia* usually absent or very sparse—present on about 1% oogonia; when present normally one to an oogonium; monoclinal (androgynous) on a long or rarely short antheridial branch, antheridial cells thin walled, tubular or clavate; apically or very rarely laterally appressed to the oogonium. *Oospheres* usually one, rarely two in an oogonium, normally, spherical but occasionally ovoid, filling the oogonium and maturing. *Oospores* subcentric; 32.5–46.5  $\mu$  in diameter, predominantly 30–37  $\mu$ .

Collected from Jalwania Pond, Gorakhpur, January 12, 1974. I wish to thank Dr. M. W. Dick, University of Reading, England, for critically examining the description and offering valuable suggestions. I also wish to thank Dr. Y. B. Singh, Principal, St. Andrew's College, Gorakhpur, for providing facilities for work.

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#### Axillary Testis in the Common House Crow (*Corvus splendens* Vieillot) and Cattle Egret (*Bubulcus ibis* Linnaeus)

While studying the annual reproductive cycle of some avian species inhabiting the semi-arid and arid tracts of Rajasthan one specimen each of the common house crow and egret was encountered which exhibited an axillary testis. Both the abnormal birds were examined in the month of July when the birds were sexually active.

In most of the birds including the crow and egret the left testis is longer than the right. However, in the abnormal birds the left testis was smaller than the right. The left axillary testis was attached independently and was in direct communication with the left vas deferens.

Histologically, the axillary testis of common house crow and cattle egret resembled in every respect with the individual testes of the abnormal specimens as well as with the testes of normal sexually active males dissected on the same day. The axillary testes of both the specimens were spermatogenetically active containing all stages of developing germ cells.

In the absence of earlier reports, the above abnormalities in the male reproductive organs of the common house crow and the cattle egret, may be considered to be purely developmental in which probably physiological rather than cytological or genetic changes are concerned.

Reproductive Physiology  
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University of Rajasthan,  
Jaipur, December 17, 1974.

D. K. VYAS.

## A Very Early Mutant in Rice

The early rice mutant reported here was obtained from the hybrid culture CR. 113-32 following gamma ray treatment (30 Kr from a 60 Co source) of dry seeds.

The mutant was 9 cm shorter in height, 18 days earlier in duration and 3 cm shorter in panicle length than the control. Another interesting change was in the shape of grain to short bold from medium slender. There was no other significant morphological change in the mutant. It was sown on 22nd June, 1974 and harvested on 28th August, i.e., a total growth period of 68 days. During this period, the rainfall at Cuttack was 492 mm.

Induction of earliness through mutation has been reported by many workers earlier. Recently Miah and Bhatti<sup>1</sup>, Saini and Gajenja<sup>2</sup>, Roy and Jana<sup>3</sup> and Miah *et al.*<sup>4</sup> have reported mutants which are 11-28 days earlier than controls. Simon<sup>5</sup> has reported mutants in rice which are 49 days earlier than its control. According to Yamagata<sup>6</sup>, mutations for heading time were more frequent than even chlorophyll mutations with gamma irradiation. In the present instance, the reduction in duration is eighteen days. This mutant was grown under completely upland condition on a sandy loam and gave 256 gm from 1.2 sq. meter or a computed yield of a little over 2 tonnes/ha. The duration (seed to seed) of this mutant in Cuttack was 80 days when transplanted in January, 68 days when direct seeded in June and 85 days when transplanted in September. This is possibly the earliest induced productive major cereal on record.

The authors thank Dr. S. Y. Padmanabhan, Director, Central Rice Research Institute, Cuttack, for facilities accorded.

Division of Genetics,  
Central Rice Res. Inst.,  
Cuttack-6 (Orissa), India,  
October 16, 1974.

C. GANGADHARAN,  
R. N. MISRA.

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6. Yamagata, H., *Gamma Field Symp.*, 1964, 3, 31.

## Isolation of Mycoplasmas from Pneumonic Lungs of Pigs in India

Goodwin *et al.*<sup>1</sup> and Mare and Switzer<sup>2</sup> isolated mycoplasma organisms in cell free medium from cases of enzootic pneumonia in pigs and called them as *M. suis* and *M. hyopneumoniae* respectively. The occurrence of mycoplasma in the Indian swine is reported for the first time.

The pneumonic and normal lung tissue from sick and healthy pigs were collected from a Bacon Factory where pneumonia of enzootic nature was reported<sup>3</sup>. The procedure of isolation of mycoplasma from these materials is given by Goodwin *et al.* From the eight pneumonic lungs, mycoplasma organisms were isolated on solid medium. The size of the colonies of mycoplasma on solid medium varied from about 20  $\mu$ -150  $\mu$  in diameter after incubating them for three days at 37°C in moist atmosphere, containing 5-10% CO<sub>2</sub> tension in 'Mc Intosh and Fildes' Jar. The small velvety colonies of mycoplasma were transparent having a characteristic 'fried egg' appearance and central elevation was not prominent in majority of them. The morphology and the growth characters of colonies were suggestive of *M. hyopneumoniae*. No mycoplasma could be isolated from six normal lungs.

Further studies on characterization of the different mycoplasma isolates and experimental reproduction of the disease are in progress. This is the first reported instance of isolation of mycoplasma from pneumonic lungs of pigs in India.

The authors thank the staff of C.D.F., Aligarh, for help in collection of the material and Principal Sri. C. V. G. Choudary for providing the facilities. Dept. of Bacteriology, U.P. College of Veterinary Science and Animal Husbandry, Mathura, December 25, 1973.

1. Goodwin, R. F. W., Pomeroy, A. P. and Whittlestone, P., *Vet. Rec.*, 1965, 77, 1247.
2. Maré, C. J. and Switzer, W. P., *Vet. Med.*, 1965, 60, 841.
3. Asthana, V. S. and Saxena, *Proc. XIV Conference Res. An. Dis., India*, 1972.

## REVIEWS AND NOTICES OF BOOKS

**Annual Review of Plant Physiology (Vol. 25).**  
Edited by Winslow R. Briggs, Paul B. Green and Russell L. Jones. (Annual Reviews, Inc., 4139, El Camino Way, Palo Alto, California 94306). 1974. Pp. 627. Price: U.S.A. \$ 12.00; Foreign \$ 12.50.

In this volume of *Annual Review of Plant Physiology* the following two review articles in the area of Cell organization in relation to function have appeared: (1) The  $C_4$  syndrome: A structural analysis by W. M. Laetsch and (2) Microtubules and microfilaments by P. K. Hepler and B. A. Palevitz. Under Nutrition, Absorption and Transport, one article on Uptake mechanisms (Inorganic and organic) by Per Nissen and another on Phloem transport Physical, Chemical or Impossible by I. F. Wardlaw are written. Two reviews one on: Metabolite exchange between chloroplasts and cytoplasm by U. Heber and another on Energy conservation in photosynthetic electron transport of chloroplasts by A. Trebst under Bioenergetics are included.

In the general topic of Metabolism the following five articles reviewing the different subjects are given: (1) Dinitrogen fixation by M. J. Dilworth, (2) Circadian rhythms and metabolic patterns by O. Queiroz, (3) Protein turn-over in plants and possible means of its regulation by R. C. Huffaker and L. W. Peterson, (4) The metabolism of aromatic compounds by H. A. Stafford, (5) Metabolism of auxin in higher plants by E. A. Schneider and F. Wightman.

In the field of development the five articles are: (1) Plant propagation through tissue culture by T. Murashige, (2) Control of seed germination by A. M. Mayer and Y. Shain, (3) Rapid responses to plant hormones by M. L. Evans, (4) Isozymes in development and differentiation by J. G. Scandalios, (5) The chemistry and physiology of abscisic acid by B. V. Milborrow are written.

Two special topics, Phytotoxins produced by Plant Parasites by G. A. Strobel and Physiology of Mycorrhiza by F. H. Mayer are reviewed.

The prefatory chapter of this volume by F. W. Went entitled "Reflections and speculations" makes an interesting reading. The rich experience of Dr. F. W. Went in the field of auxinology and plant environmental problems are very succinctly reported.

K. S. KRISHNA SASTRY.

**Biology Digest.** Published by Data Courier, Inc., 620, South Fifth Street, Louisville, Kentucky 40202, U.S.A. Subscription \$ 75 per year, Vol. I, Issue 1, Pp. vii + 199. September 1974.

*Biology Digest* is a new monthly information service launched to organize, summarise and index worldwide scientific literature in the life sciences. The Digest is designed primarily to assist students and educators at the Secondary School and Undergraduate College levels in keeping current on latest scientific developments through abstracts and feature articles.

A subject of great topical interest 'What we know about cancers' is the feature article of this first issue. The Abstracts sections cover the areas of Plant life, Living systems, Micropopulations, Biosphere, Biogenesis and Development, Animal kingdom and Health science. M. SIRSI.

### ANNOUNCEMENTS

#### **Hari Om Ashram Vikram Sarabhai Research Awards for the Year 1974**

Hari Om Ashram Vikram Sarabhai Research Awards for the year 1974 have been awarded as follows:

I. Electronics and Telecommunications: Jointly to Dr. S. Krishnan, National Aeronautical Laboratory, Bangalore and Dr. S. Srikantan, Electronics Corporation of India, Hyderabad; II. Planetary and Space Sciences; Prof. Satya Prakash, Physical Research Laboratory, Ahmedabad; III. Atmospheric Physics and Hydrology: Dr. R. N. Keshavamurthy, Meteorological Department, Poona; IV. Systems Analysis and Management: Dr. N. Seshagiri, Electronics Commission, New Delhi.

#### **ĀRŌGYA—A JOURNAL OF HEALTH SCIENCES**

ĀRŌGYA—A Journal of Health Sciences (Half Yearly) will be published by The Academy of General Education under the Editorship of Prof. J. V. Bhat, Emeritus Scientist, Kasturba Medical College, Manipal. This Journal will disseminate authentic and reliable information on all aspects of Health Sciences as Medical, Dental, Pharmaceutical, Nursing and other Borderline areas as Bio-medical Engineering, Sociology, etc., etc.

Further details can be had from Prof. J. V. Bhat, Editor, ĀRŌGYA, Kasturba Medical College, Manipal 576119.



## INFORMATION TO CONTRIBUTORS

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- (iv) Short Scientific Notes—not exceeding 250 words.

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References are indicated by superscripts in the text. The style of references should be *Journal*: The names of the authors, the journal, year, volume and page.

*Book*: Authors names, the title of the book, name and location of the publishers, year and page.

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# ROLE OF $f$ -GRAVITY IN COSMOLOGICAL MODELS

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## ABSTRACT

The role of a massive scalar-tensor theory of  $f$ -gravity in cosmology is clarified to remove earlier ambiguities.

IN recent papers<sup>1,2</sup> we investigated the cosmological consequences of incorporating  $f$ -gravity (i.e., the short-range strong gravity field mediated by massive spin-2  $f$ -mesons with its coupling constant  $G_f$  of strength of the order of the strong interactions) together with a cosmological constant  $\Lambda_f$  of the order of magnitude of the square of the inverse Compton wavelength of the  $f$ -meson to take account of the short range. The purpose of the present note is to clarify a misleading ambiguity in that work, and to extend the discussion to cosmological models with spaces of non-zero curvature.

The Robertson-Walker (R-W) metric with flat 3-space ( $K=0$ ) is determined by the equation:

$$\dot{R}^2 = \frac{2GM}{R} + \frac{2}{3} \Lambda_f c^2 R^2 \quad (1)$$

where  $M = (4\pi/3) \rho R^3 = \text{total mass} = \text{a constant}$ . The  $\Lambda$ -term occurs with a positive sign, so that a *minimum* in the function  $R(t)$  will occur only if the coupling constant  $G_f$  of  $f$ -gravity that replaces the Newtonian constant (at short ranges) is *negative* and  $\Lambda_f$  *positive*. This was not made clear in references 1 and 2, because the  $\Lambda$ -term appeared with a negative sign.

The solution of equation (1) is:

$$R = (3 |G_f| M / \Lambda_f c^2)^{1/3} \cosh^{3/2} \left( t \sqrt{\frac{3 \Lambda_f c^2}{2}} \right) \quad (2)$$

This has a *minimum* at:

$$R_{\min} = (3 |G_f| M / \Lambda_f c^2)^{1/3} \quad (3)$$

With  $M$  being the total mass of the presently observable universe corresponding to  $\sim 10^{80}$  baryons. Using  $G_f$  as given in reference 3 and  $\Lambda_f$  as given in reference 4, we obtain:

$$R_{\min} \sim 10^{13} \text{ cm},$$

corresponding to a maximum density of

$$\rho_{\max} \sim \Lambda_f c^2 / 4\pi |G_f| \sim 10^{17} \text{ g.cm}^{-3},$$

as stated in references 1 and 2.

For  $K = \pm 1$ , the R-W line-element implies (i.e., for a curved 3-space):

$$\dot{R}^2 = \frac{2GM}{R} + \frac{2 \Lambda c^2 R^2}{3} \mp c^2 \quad (4)$$

For  $K = +1$ , and  $0 < \Lambda < C^2/3GM$ , the cosmology has no singularity anyway, so the question of invoking  $f$ -gravity does not arise. If the inequality concerning  $\Lambda$  is not satisfied, or if  $K = -1$ , we have to explore the possibility that the singularity is averted by the effects of  $f$ -gravity coming into play at high densities. Replacing the Newtonian  $G$

by  $G_f$  (negative) and  $\Lambda$  by  $\Lambda_f$ , we find a minimum value of  $R$  at

$$\begin{aligned} \sqrt{\frac{2}{\Lambda_f}} \cosh \left( \frac{1}{3} \cosh^{-1} A \right), & \quad (K = +1) \\ \sqrt{\frac{2}{\Lambda_f}} \sinh \left( \frac{1}{3} \sinh^{-1} A \right), & \quad (K = -1) \end{aligned} \quad (5)$$

where

$$A = 3 |G_f| M \sqrt{2 \Lambda_f} / c^2 \sim 10^{81} \quad (6)$$

As  $A$  is large, both the expressions (5) are approximately equal to the expression (3) for  $R_{\min}$ . Hence the maximum density is the same as for the case  $K=0$ . We emphasize that with negative  $G_f$  the  $\Lambda_f$  term is *essential* if  $K=0$  or  $+1$ , since with  $\Lambda_f=0$ , these equations have no real solutions.

The sign of  $G$  in general relativity is fixed by requiring that Newton's gravitational theory shall correspond to the linearised theory. However, to avert the singularity by incorporating  $f$ -gravity requires a repulsive short-range force, i.e., opposite sign of  $G_f$ . This naturally leads to the question as to how a theory with the repulsive effect necessary to avoid the cosmological singularity is consistent with recent work by two of the present authors (C. S. and K. P. S.) wherein  $f$ -gravity can also provide the strong binding forces for elementary particles. An answer to this is to be found in a recent paper of the authors in which the field equations of the short range massive  $f$ -gravity (with "cosmological" constant  $\Lambda_f$ ) is shown to give rise to a massive scalar-tensor theory. The ratio of the masses of the mediating particles is  $m_0$  (scalar)/ $m_2$  (tensor)  $= \sqrt{3}$ , indicating that the scalar has a shorter range. Furthermore, the tensor is found to be attractive (as in ordinary general relativity) whereas the scalar is a repulsive potential dominating at still shorter distances and higher densities. It is this repulsive interaction which averts the cosmological collapse. Under such conditions of high densities the weak Newtonian gravity (which is always attractive) loses its meaning.

1. Sivaram, C., Sinha, K. P. and Lord, E. A., *Nature*, 1974, 249, 640.
2. —, — and —, *Curr. Sci.*, 1974, 43, 199.
3. — and —, *Lett. Nuovo Cimento*, 1974, 9, 704.
4. — and —, *Ibid.*, 1973, 8, 324.
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6. Lord, E. A., Sinha, K. P. and Sivaram, C., *Progr. Theoret. Phys. (Kyoto)*, 1974, 52, 161.

## SUPERCONTINENTS OVER THE GEOLOGICAL TIMES

P. C. PAUL

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## ABSTRACT

A palaeomagnetic polar wander curve relative to India is prepared for the past 1600–1800 m.y. period. Comparison of this and the corresponding African curve suggests that Gondwanaland existed only about 100 m.y. ago. The palaeomagnetic data thus suggest that the history of the global surface since the middle Tertiary times has been characterised by the existence of three supercontinents during different periods—the Afro-American supercontinent from about 1800 m.y. ago, Gondwanaland from late Proterozoic till late Mesozoic, and the Eurasian supercontinent since the Tertiary times.

## 1. INTRODUCTION

ONCE crucial question in the postulate of global plate tectonics<sup>1,2</sup> is whether the large scale plate motions, known to have occurred since the late Mesozoic, also took place in the older times. The palaeomagnetic evidence strongly supports this possibility<sup>3,4</sup>. The palaeomagnetic data from Africa and the South and North Americas, for instance, suggest that these three continents were joined together in a single supercontinent until about 1600 m.y. ago<sup>5</sup>. Since the European data seem to contradict the existence of Pangaea in the Precambrian times, the question now arises whether this Afro-American assembly existed as part of a 'Greater Gondwanaland' or as a separate supercontinent. This is examined here from the palaeomagnetic data for the entire Proterozoic-Proterozoic interval which are now available for India, summarised below, Australia<sup>6,7</sup>, Africa<sup>8,9</sup> and South America<sup>4</sup>.

## 2. THE BIRTH OF GONDWANALAND

The palaeomagnetic poles obtained from the Indian rock formations are summarised in Table I and the corresponding polar wander curve is shown in Fig. 1. The Precambrian part of this is the modification of an earlier<sup>10</sup> polar wander curve and all the available data have been included. The Phanerozoic part of this curve is based on an earlier summary<sup>10</sup> and incorporates the new data<sup>11</sup> available since then. In view of the detailed magneto-stratigraphic correlations<sup>12,13</sup> and agreement with the marine-magnetic<sup>14</sup> and palaeoclimatic<sup>15</sup> deductions, this can be considered to be a fairly complete polar wander curve for India for the past 1600 to 1800 m.y. duration.

The Phanerozoic palaeomagnetic data from India and the other Gondwanic continents<sup>6,7,16</sup> are consistent with the Gondwanic reconstructions of du Toit<sup>17</sup> and Smith and Hallam<sup>18</sup>. These reconstructions can therefore be used to study the older palaeomagnetic data from these continents.

Accordingly, the Indian polar wander is recomputed<sup>19</sup> for the India-Africa assembly of the latter reconstruction and compared with the corresponding African curve in Fig. 2. This comparison suggests that India and Africa had not assembled in their early and middle Phanerozoic Gondwanic position until about 75–800 m.y. ago. These data also fail to support Piper's<sup>20</sup> claim that the Precambrian

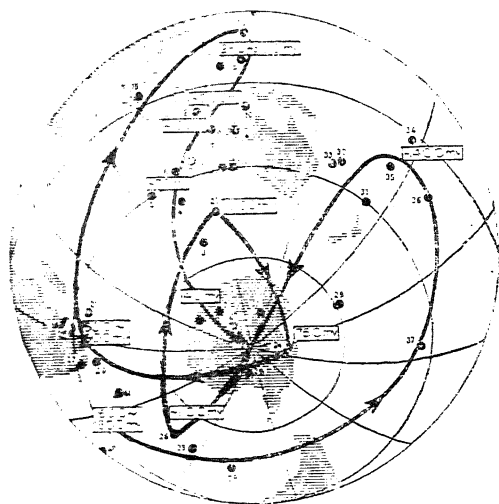


FIG. 1. Palaeomagnetic polar wander (south pole) track for India. 1: Siwalik redbeds; 2–5: Deccan traps; 6–7: Tirupathi and Satyavedu sandstones; 8–9: Rajmahal and Sylhet traps; 10–13: Pachmarhi, Mangli, Parsora and Himgir sandstones; 14–16: Kamthi beds; 17: Talchir series; 18: Speckled sandstones; 19: Salt Pseudomorph beds; 20: Purple sandstones; 21–22: Rewa and Bhandar sediments; 23: Kondapalle charnokites; 24: Malani rhyolites; 25: Mundwara complex; 26: Lower Sullavals; 27: Kaimur sandstones; 28: Upper Pekkals; 29–30: Bhima sediments; 31: Lower Pekkals; 32–33: Cuddapah sediments; 34–35: Lower Vindhya (B.H.Q. and B.H.J.); 36: Visakhapatnam charnokites-I; 37–38: Horahelli and Chitaldurg dykes; 39: Visakhapatnam charnokites-II; 40: Gwalior lavas; 41: Hyderabad dyke.

India, Africa and Australia came together in the Gondwanic assembly only in the late Proterozoic times. As has been stated earlier, the palaeomagnetic data also suggest that South America and Africa were already joined together at this time<sup>4</sup>.

TABLE I

| Geological interval and age | Rock suites  | Mean pole (south) |           |
|-----------------------------|--|-------------------|-----------|
|                             |  | Latitude          | Longitude |
| Tertiary                    | Siwalik red beds and Deccan lavas  | 63° S             | 85° E     |
| Late Cretaceous             | Deccan lavas and upper Gondwana sediments                                  | 27° S             | 166° E    |
| Early Cretaceous-Jurassic   | Rajmahal and Sylhet lavas  | 12° S             | 118° E    |
| Permian-Triassic            | Lower Gondwanas and Speckled sandstones                                    | 17° S             | 125° E    |
| Permian-Carboniferous       | Kamti and Talchir beds   | 27° N             | 132° E    |
| Cambric-Late Proterozoic    | Salt Range and upper Vindhyan sediments and Charnockites                   | 32° S             | 30° E     |
| About 750 m.y.              | Malani rhyolites   | 78° S             | 225° E    |
| 950-1000 m.y.               | Mundwara complex   | 43° S             | 116° E    |
| About 1100 m.y.             | Lower Sullavai sediments   | 49° S             | 341° E    |
| About 1150 m.y.             | Kaimur and upper Pakhal sediments  | 81° S             | 204° E    |
| 1200-1300 m.y.              | Bhima sediments  | 68° S             | 172° E    |
| About 1400 m.y.             | Lower Pakhal, lower Cuddapah and lower Vindhyan sediments and Charnockites | 19° S             | 165° E    |
| 1600-1800 m.y.              | Charnockites, dykes and Gwalior lavas                                      | 37° S             | 346° E    |

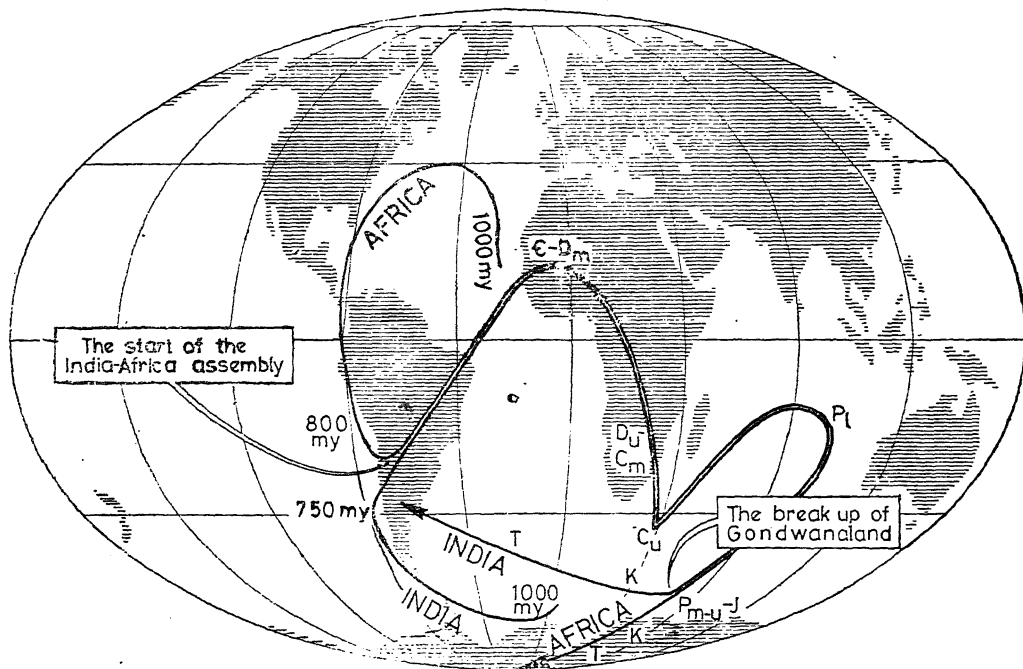


FIG. 2. Comparison of the Indian and African palaeomagnetic polar wander curves. The Indian data, summarised in Fig. 1, recalculated for the India-Africa assembly of the Gondwanic times, i.e., 58.9° clockwise rotation of India, with respect to Africa, with the rotation pole at 28.9° N; 42.2° E. The African polar wander curve is after Brock and Piper<sup>16</sup>.

### 3. AFRO-AMERICAN, GONDWANIC AND EURASIAN SUPERCONTINENTS

The Gondwanic supercontinent obviously resulted from the type of convergent plate motions of which Eurasia<sup>2,22,23</sup> is a latter-day example. The history of Gondwanaland can thus be considered in three stages: (i) the late Proterozoic formative stage of convergent plate motions, (ii) the approximately 700 m.y. long supercontinent stage, and (iii) the late Mesozoic fragmentation stage of divergent plate motions. The inference drawn above suggests that the Afro-American supercontinent<sup>4</sup> fragmented, with the separation of North America about 1000 m.y. ago, long before the Gondwanic supercontinent came into being. This is analogous to the later (late Mesozoic) break-up of the latter which preceded the formation of the Eurasian supercontinent. Another common feature of the three supercontinents (Table II) is their crustal volume (about 2.5 billion Km<sup>3</sup>).

feature in the evolution of the global surface. It will be quite interesting, therefore, to examine whether the supercontinents should be considered to be of intrinsic importance in the framework of the plate tectonics theory. Studying plate interactions in terms of the Feynman graphs for electron-photon scattering in quantum-electrodynamics appears to be a promising attempt<sup>24</sup> in this direction.

The concentrations of the continental crust in the form of supercontinents, as suggested in the foregoing, does not seem to be an exclusively terrestrial phenomenon however. The Martian surface<sup>25</sup>, for instance, is also characterised by a similar feature, i.e., only one supercontinent of rather comparable dimensions.

### 5. ACKNOWLEDGEMENTS

I thank Professor V. L. S. Bhimasankaram for suggesting this study and for many helping discus-

TABLE II  
*Terrestrial supercontinents since middle Proterozoic*

| Geological Interval  | Supercontinent and the constituent continents                                 | Hemisphere occupied | Area (10 <sup>6</sup> Km <sup>2</sup> ) | Crustal volume* (10 <sup>9</sup> Km <sup>3</sup> ) |
|--|---|---------------------|---|--|
| Since Tertiary   | <i>Eurasia</i><br>Europe, Siberia, China, India and Arabia                    | Northern            | 55                                      | 2.5  |
| Late Mesozoic-Late Proterozoic   | <i>Gondwanaland</i><br>South America, Africa, India, Australia and Antarctica | Southern            | 74                                      | 2.6  |
| Till the close of middle Proterozoic times, i.e., till about 1000 m.y. ago | <i>Afro-America</i><br>Africa, South America and North America                | Southern            | 72                                      | 2.5  |

\* Average crustal thickness assumed about 45 Km for Eurasia and about 35 Km for the other two supercontinents.

The continental concentrations on much larger scales also appear to have occurred in the geological past. The palaeomagnetic evidence<sup>3</sup> suggests that Pangaea, with a crustal volume of about 4 billion Km<sup>3</sup>, existed from the Silurian till the Triassic times. This resulted from the merger of Euramerican and Gondwanic land-masses during this period. In case Siberia indeed joined Europe in the Permo-Triassic<sup>3,22,23</sup>, then the Triassic Pangaea must have had the maximum crustal volume (about 4.5 billion Km<sup>3</sup>) amongst the supercontinents that have existed on the global surface during the past billion years. Piper *et al.*<sup>4</sup> have also speculated on the possibility of the concentration of all continental crust in one large mass in the middle Proterozoic times.

### 4. CONCLUSIONS

These observations suggest that the growth and dispersal of the supercontinents have been a major

sions. I have also profited greatly from discussions with Dr. S. M. Naqvi.

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## THE ACTION OF CHLORPROMAZINE ON THE SKELETAL MUSCLE OF FROG

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### ABSTRACT

The action of Chlorpromazine on the skeletal muscle of frog when tested under Gaddum superfusion technique was found to be (1) an anticholinergic effect in small doses and (2) a spasmogenic effect in large doses, this spasmogenic effect was not blocked by curare.

### INTRODUCTION

CHLORPROMAZINE (CPZ) is one of the phenothiazine derivatives widely used in psychiatry and also in the treatment of spastic conditions. The untoward effects of this drug include parkinsonian like symptoms and dyskinetic symptoms which may be mistaken for tetanus, meningitis, poliomyelitis, etc. These symptoms are explained as the effect of the drug on the extrapyramidal system. This work was taken up to find the effect of graded concentration of CPZ on the skeletal muscle.

### MATERIALS AND METHOD

Experiments were carried out on the skeletal muscle of frog *Rana tigrina* under Gaddum superfusion technique<sup>4,5</sup>. The ringer solution prepared according to Burn J. H. (1952) was used. The contractions were recorded on a slow moving smoked drum. Acetylcholine chloride (ACH) was used as an agonist in a dose of 1–2 mcg.

CPZ dissolved in distilled water was used in doses of 0.1, 1, 10, 100 ng<sup>+</sup> and 1, 10 and 100 mcg

in 0.1 ml volume. The effect of CPZ in higher doses like 100 mcg and above were recorded on a stopped drum. In these experiments ACH was not used as an agonist, and they were repeated in potassium-free, calcium-free, sodium-free ringer solution and the solution containing twice the concentration of potassium ion. Tubocurarine 10 mg/ml was used as a blocking agent.

All drugs were dropped from a tuberculine syringe along with ringer solution. The contractions due to ACH were recorded for 20 seconds. After obtaining a set of submaximal contractions due to ACH, the CPZ was dropped followed by ACH after 20 seconds. The inhibition or potentiation of ACH induced contractions by CPZ was expressed as the height of contraction in mm.

### RESULTS

The effects of CPZ on skeletal muscle can be grouped under three headings.

1. Anticholinergic action in doses of 0.1 ng to 10 mcg (Fig. 1).
2. Potentiation of ACH induced contraction in 0.1 ng and 1 ng.
3. A spasmogenic action at 100 mcg and above (Fig. 2).

\* Former Professor and Head of the Department.



TABLE I

Showing the anticholinergic action of CPZ expressed as percentage inhibition of ACH induced contractions in 20 experiments

|                                   | 0.1 ng*         | 1 ng            | 10 ng            | 100 ng         | 1 mcg           | 10 mcg          |
|-----------------------------------|-----------------|-----------------|------------------|----------------|-----------------|-----------------|
| % inhibition $\pm$ standard error | 27.8 $\pm$ 2.34 | 19.5 $\pm$ 0.81 | 24.08 $\pm$ 1.00 | 16.4 $\pm$ 1.2 | 55.9 $\pm$ 1.55 | 48.34 $\pm$ 2.0 |

\*ng = nanogram.

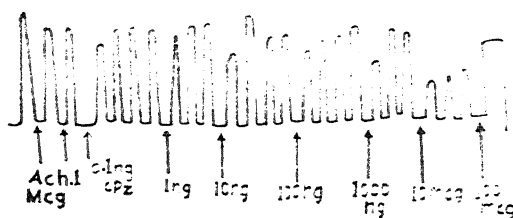


FIG. 1. Showing anticholinergic action of CPZ in doses of 0.1 ng to 10 mcg, each dose of CPZ is followed by 1 mcg of ACH except at 100 mcg.

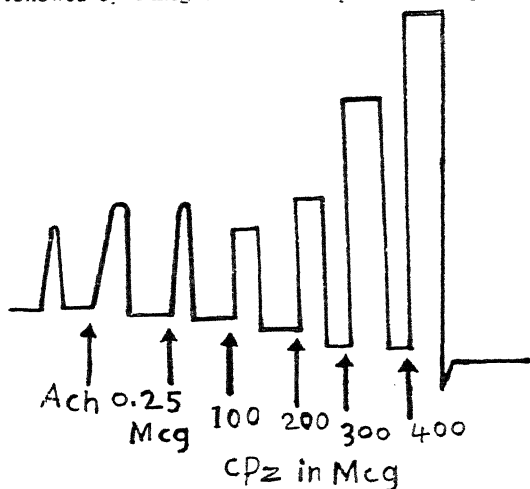


FIG. 2. Showing the spasmogenic action of CPZ in 100, 200, 300 and 400 mcg, ACH is not used after CPZ.

1. CPZ in doses from 0.1 ng to 10 mcg produced a dose-dependent block of ACH induced contractions on skeletal muscle. The block is directly proportional to the dose at 100 ng to 10 mcg.

2. In 7 out of 20 experiments CPZ in doses of 0.1 ng and 1 ng produced either an immediate or a delayed potentiation of  $17.2 \pm 2.47\%$  of ACH induced contraction of the skeletal muscle. In one out of these 7, the potentiation lasted during the entire course of the experiment, whereas in others it lasted for a short time.

3. *Spasmogenic action*: CPZ when used at 100 mcg and above produced a classic phasic contracture of the skeletal muscle which develops very slowly in over 20-30 minutes. During the first few

minutes the tissue does not exhibit any effect, then slowly, an upward deflection reaching a maximum height of 30-70 mm depending upon the dose. The tissue fails to recover completely even after 6 hours, during which, it remains insensitive to ACH also. This action of CPZ was not blocked even by 200 mcg of curare.

*In ion-free solutions*: In calcium-free solution CPZ failed to produce the spasmogenic effect. In potassium-free solution CPZ produced the contracture of a lesser height and intensity and the tissue recovered on gently pressing down the lever in 40-60 minutes and responded in a graded fashion to the increasing dose of CPZ.

In sodium-free Ringer and Ringer solution containing double the concentration of potassium ion, the tissue developed a contracture.

#### DISCUSSION

The substituted phenothiazines have multiple action on peripheral autonomic nervous system. These include the blockade of nicotinic and muscarinic action of ACH<sup>8</sup>. CPZ is said to produce the relaxation of skeletal muscle in some types of spastic conditions by selectively acting on the gamma efferent system and does not produce a blockade of the neuromuscular junction<sup>2,9</sup>. But a perusal of Table I shows that it blocks the action of ACH on skeletal muscle. The rectus abdominis muscle of frog is one of the preparations used to demonstrate the action of drugs at neuromuscular junction<sup>4</sup>. CPZ produces a curare-like action on the skeletal muscle. In these experiments the anticholinergic action is  $27.77 \pm 2.34$  at first exposure of the tissue to the drug, whereas in next two exposures it is  $19.5 \pm 0.81$  and  $24.08 \pm 0.999$  though the dose of the drug is increased in a graded fashion. This may be because the tissue may show a high initial sensitivity when exposed to the drug for the first time.

The CPZ has also shown a potentiation on the action of ACH on skeletal muscle in dose of 0.1 ng and 1 ng. CPZ is reported to enhance the seizures after its depressant action of a large dose wears off<sup>8</sup>. Also CPZ is said to be an anticholinesterase agent<sup>9</sup>. Therefore the potentiation of ACH action on the skeletal muscle is probably mediated by the anticholinesterase action of the drug.

It may be seen from Fig. 2 that CPZ produces a classic phasic contracture of the skeletal muscle. The fact that it is not blocked by curare, a competitive blocker of the neuromuscular junction and CPZ itself produce a neuromuscular blocking action, shows that the drug produces contracture by acting at a receptor site other than the neuromuscular junction. The next alternative site of action would be the cell membrane or the contractile machinery. The addition of high concentration of potassium to the isolated muscle initiates the classic phase of potassium contracture, but it is not necessarily correlated with the contractility<sup>10</sup>. In potassium-free ringer CPZ produces contracture but relaxes quickly compared to the effect in normal ringer. Its failure to produce this effect in calcium-free ringer suggests that this action is mediated through potassium and calcium ions. CPZ is reported to be having a membrane stabilising action<sup>6</sup>, this demonstrates that the contracture is produced by stabilising the cell membrane, so as to rise the tissue concentration of potassium and calcium ions beyond physiological limits, and more so in a rise of potassium resulting in contracture.

Thus, CPZ is shown to have an anticholinergic action in low doses and a membrane stabilising action resulting in contracture of the skeletal muscle of frog in high dose,

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### RADIOCARBON DATES OF SOME LATE QUATERNARY SAMPLES

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PRESENTED below are the <sup>14</sup>C dates of Late Quaternary samples from the coastal and riverine sediments. The eustatic samples are derived from the western coast. Quite a few samples of miliolite formations of Gujarat have also been dated. The river sediments have been <sup>14</sup>C dated for their Stone Age associations.

The samples have been counted in the form of methane in gas proportional counters. The techniques have been described in detail elsewhere (Agrawal and Kusumgar, 1965; Agrawal *et al.*, 1971; Kusumgar *et al.*, 1963). Ninetyfive per cent activity of N.B.S. oxalic acid has been used as a modern standard. For all samples two dates are given in B.P. The first is based on 5568 yr. half-life value and the second, in parenthesis, on 5730 yr. None of the dates has been calibrated for any <sup>14</sup>C/<sup>12</sup>C variations. The dates can be converted to AD/BC scale by using 1950 AD as reference year,

Though CaCO<sub>3</sub> measurements too have been expressed in terms of <sup>14</sup>C dates, yet it has to be noted that CaCO<sub>3</sub> is an inorganic chemical and the <sup>14</sup>C method, strictly speaking, does not apply to it.

These measurements were made at the Tata Institute, Bombay, but now the C<sup>14</sup> lab has been shifted to Physical Research Laboratory, Ahmedabad.

#### ACKNOWLEDGEMENTS

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Asla, Maharashtra India

TF-1178, Late Quaternary, 9750 ± 120  
(10035 ± 125)

Shells from pebbly conglomerate 2.5 m above Krishna river bed at Asla (Lat. 17° 53' N., Long. 73° 59½' E.), District Satara,

**Barda Hill, Gujarat, India**

Mollitic limestone from a running quarry near Barda (Lat.  $21^{\circ} 35' N.$ , Long.  $69^{\circ} 55' E.$ , District Porbandar).

TF-764 (a), Late Quaternary,  $17915 \pm 415$   
(14415  $\pm$  445)

Whole sample was measured after repeatedly washing with 1 normal HCl.

TF-764 (b), Late Quaternary,  $12990 \pm 185$   
(13170  $\pm$  199)

Oolitic grain fraction ranging between 150 to 420 microns: washed repeatedly with 1 normal HCl.

**Beli Iena athula, Ceylon**

TF-1094, Prehistoric Cave,  $7690 \pm 110$   
(7660  $\pm$  119)

Carbonised kernels collected at a depth of 45 m from a prehistoric cave (Lat.  $6^{\circ} 56' 5'' N.$ , Long.  $80^{\circ} 14' 5'' E.$ , near Maniyanguma, 4 km S.E. of Avisawella).

**Cochin Harbour Area, Kerala, India**

TF-1147, Late Quaternary,  $8795 \pm 115$   
(9650  $\pm$  115)

Log of wood found at the depth of 21 m during well sinking from Cochin harbour area (Lat.  $9^{\circ} 57' N.$ , Long.  $76^{\circ} 15' E.$ , District Ernakulam).

**Continental shelf, West Coast, India**

The samples were collected by dredging on the continental shelf.

TF-958, Late Quaternary,  $8640 \pm 130$   
(9200  $\pm$  135)

Oolitic limestone from continental shelf off Bombay (Lat.  $18^{\circ} 36' N.$ , Long.  $70^{\circ} 39' 4'' E.$ , water depth 96 m., sample No. 42 (a)).

TF-970, Late Quaternary,  $10725 \pm 400$   
(11025  $\pm$  400)

Massive Oolitic limestone (carbonate) from continental shelf off Bombay (Lat.  $19^{\circ} 15' N.$ , Long.  $69^{\circ} 45' E.$ , water depth 150 m., sample No. 2).

TF-1299, Late Quaternary,  $19120 \pm 250$   
(19415  $\pm$  260)

Coral from continental shelf off Ratnagiri (Lat.  $17^{\circ} 30' N.$ , Long.  $73^{\circ} 30' E.$ , depth 165 m from sea level, Sample No. 10).

TF-971, Late Quaternary,  $11220 \pm 130$   
(11550  $\pm$  135)

Massive coral from continental shelf off Bombay (Lat.  $19^{\circ} 15' N.$ , Long.  $69^{\circ} 45' E.$ , water depth 150 m., Sample No. E).

**Deoghat, U.P., India**

TF-1245, Late Quaternary,  $19155 \pm 330$   
(19715  $\pm$  340)

Shells extracted from gravel III at Deoghat (Lat.  $24^{\circ} 54' N.$ , Long.  $82^{\circ} 2' E.$ , on River Belan, District Allahabad).

**Dhom Dam, Maharashtra, India**

TF-1114, Late Quaternary,

$11470 \pm 140$  (11590  $\pm$  9200)  
 $4125 \pm 425$  (4245  $\pm$  4245)

Shells from lenticular body of pebble conglomerate at a depth of 19 m from terrace surface on River Krishna near Dhom Dam (Lat.  $17^{\circ} 58' N.$ , Long.  $73^{\circ} 52' E.$ , District Satara).

**Dado Hill, W. Rajasthan, India**

TF-1215, Late Quaternary,  $14080 \pm 170$   
(14485  $\pm$  170)

Calcium carbonate samples from the concretionary layer of rhyolite zone of weathering on the piedmont slope. Sample No. 9.

**Gargaon, Maharashtra, India**

TF-1111, Late Quaternary,  $10020 \pm 150$   
(10310  $\pm$  155)

Calcified bones from the silt deposit, 12 m above water level and overlying the Middle Stone Age (M.S.A.) bearing gravel near Gargaon on River Mula, District Poona.

**Inamgaon, Maharashtra, India**

TF-1177, Late Quaternary,  $18750 \pm 350$   
(19250  $\pm$  360)

Freshwater shells from M.S.A. tool bearing sandy pebbles conglomerate on River Ghod and 2 m above the river bed near Inamgaon (Lat.  $18^{\circ} 36' N.$ , Long.  $74^{\circ} 32' E.$ , District Poona).

TF-1093, Late Quaternary,

$21110 \pm 515$  (21725  $\pm$  630)  
 $579 \pm 579$  (585  $\pm$  585)

Shells from the sandy pebble gravel, 2 m above Ghod river bed.

**Khadir Island, Great Rann of Kutch, India**

TF-837 (b), Late Quaternary,  $> 36,000$   
( $> 37,000$ )

Oyster shells collected 3 m above MSL from Khadir Island (Lat.  $23^{\circ} 52' 30'' N.$ , Long.  $70^{\circ} 27' 30'' E.$ ).

**Kotia, Gujarat, India**

TF-759, Late Quaternary,  $7430 \pm 130$   
(7645  $\pm$  135)

Calcium carbonate samples from freshly explored station near Kotia (Lat.  $21^{\circ} 50' N.$ , Long.  $73^{\circ} 15' E.$ ).

**Kulur, Mysore, India**

TF-966, Late Quaternary,  
 $37355 \pm 5980$  (38445  $\pm$  6150)  
 $3390 \pm 3390$  (3490  $\pm$  3490)

Root of tree extracted at the depth of 14 to 17 m from Kulur (Gurpar) river (Lat.  $12^{\circ} 55' N.$ , Long.  $74^{\circ} 50' E.$ , District Mangalore. Comment: NaOH pretreatment was given. Sample dates a river bed sedimentation.

**Kutch Area, Gujarat, India**

TF-892, Late Quaternary,

$$32530 \pm 2710 \quad (33480 \pm 2790) \\ - 2025 \quad - 2085$$

Miliolite shells collected at a depth of 7.6 m from a limestone bed near Katral hill 13 km from Bhuj Mandvi Road, District Kutch (Field No. 11/92).

TF-893, Late Quaternary,

$$28595 \pm 1600 \quad (29430 \pm 1650) \\ - 1345 \quad - 1380$$

Miliola tests from the surface exposure of the limestone bed at Katral hill, District Kutch. (Field No. 11/26).

TF-897, Late Quaternary,  $12280 \pm 165$   
( $12640 \pm 170$ )

Miliola tests collected from the surface exposure on Bhuj-Naliya road near Drubya hill, District Kutch (Field No. 11/78).

TF-898, Late Quaternary,

$$24760 \pm 1000 \quad (25480 \pm 1025) \\ - 885 \quad - 915$$

Miliola tests from surface exposures on the north flank of Jura hill, District Kutch. (Field No. 11/61).

TF-889, Late Quaternary,  $11130 \pm 150$   
( $11450 \pm 155$ )

Miliolite samples collected at Washtana (Lat.  $23^{\circ} 25' N.$ , Long.  $70^{\circ} 34' E.$ ), District Waga. (Field No. 11/132).

**Nicora, Gujarat, India**

Calcite samples collected from the right bank of river Narmada at Nicora (Lat.  $21^{\circ} 46' N.$ , Long.  $73^{\circ} 7' E.$ ), District Broach.

TF-900, Late Quaternary,  $16825 \pm 225$   
( $17315 \pm 230$ ).

Calcite sample No. 2.

TF-901, Late Quaternary,  $17810 \pm 290$   
( $18330 \pm 300$ ).

Calcite sample No. 3.

**Panambur, Mysore, India**

TF-1089, Late Quaternary,

$$37375 \pm 4960 \quad (38460 \pm 5105) \\ - 3100 \quad - 3190$$

Carbonised wood from ancient coastal sediments at a depth of 11.5 m to 12 m near Punalur Harbour area (Lat.  $12^{\circ} 56' N.$ , Long.  $74^{\circ} 50' E.$ ), District South Kanara.

**Patan, Gujarat, India**

TF-1047, Late Quaternary,  $19645 \pm 315$   
( $20215 \pm 325$ )

Shells from a 50 m thick exposed bed near Patan, District Junagarh. Stratigraphy not clear. (Field No. LOC-7.6/1969).

**Pokran, W. Rajasthan, India**

TF-1214, Late Quaternary,

$$27875 \pm 1985 \quad (28690 \pm 2045) \\ - 1605 \quad - 1650$$

Carbonate sample from concretionary layer about 1 m below aeolian sand, 15 km east of Pokran.

**Rati Karar, M.P., India**

TF-967, Late Quaternary

$$32750 \pm 1770 \quad (33700 \pm 1820) \\ - 1580 \quad - 1625$$

Shells collected at a depth of 11 m from a M.S.A. tool bearing and Late Pleistocene fossiliferous sandy pebbly conglomerate on River Narmada near Rati Karar village, District Narsinghpur.

**Tasgaon, Maharashtra, India**

TF-1213, Late Quaternary,  $3745 \pm 105$   
( $3855 \pm 110$ )

Wood sample 10 m below terrace level on the left bank of River Krishna near Tasgaon (Lat.  $17^{\circ} 2' 30' N.$ , Long.  $74^{\circ} 39' E.$ ), District Sangli. The deposit also yielded late Pleistocene fauna.

**Vembanad Lake, Kerala, India**

Shells extracted from the bed of Vembanad Lake to study the rate of sedimentation.

TF-1090,  $3625 \pm 95$  ( $3735 \pm 100$ )

Shells 3.35 m below the present bed. Sample No. 1.

TF-1091,  $3945 \pm 140$  ( $4060 \pm 145$ )

Shells 1.82 m below the present bed. Sample No. 2.

Samples sent by : TF-1178, -764 (a), -764 (b) -1004, -1111, -1177, -1003, -967, -1213 by Deccan College, Poona ; TF-1094 by BSIP, Lucknow ; TF-1147 by Kerala Engineering Research Inst., Peechi ; TF-968, -970, -1200, -971, -1090, -1091 by NIO, Goa ; TF-1245 by Allahabad University, Allahabad ; TF-1215, -1214 by CAZRI, Jodhpur ; TF-837 (b), -892, -898, 889 by ONGC, Baroda ; TF-759, -900, -901 by M.S. University, Baroda ; TF-966, -1089 by Karnataka Regional College of Engineering, Surathkal ; TF-1047 by TIFR, Bombay.

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## LETTERS TO THE EDITOR

## EMISSION SPECTRUM (C-X AND D-X SYSTEMS) OF THE MOLECULE—SrBr

Early investigations on the band spectrum of the diatomic molecule—SrBr had been reported by Oimsted<sup>1</sup> in bunsen flame and Hadfield<sup>2</sup> using Oxy-acetylene flame. Walters and Burrage<sup>3</sup> were the first to observe a group of eleven red degraded bands lying in the violet region. In 1942, Harrington<sup>4</sup> made a more comprehensive study of this molecule employing absorption tubes and classified the observed bands to constitute as many as four distinct systems. He could also identify their sub-heads and vibrational isotopic shifts for many of the bands. Recently, Reddy and Rao<sup>5</sup>, using high frequency discharge source, reported yet another additional system, the E-system, lying in the spectral region  $\lambda\lambda 3000-3200$ .

In the present investigation the band systems lying in the violet and ultraviolet region for this molecule have been recorded in thermal emission, for the first time, using high temperature graphite tube furnace. Practically all of these systems of bands have been fairly extended apart from reporting quite a large number of additional stray bands.

A small quantity of pure solid strontium bromide was placed in the combustion tube of the high temperature vacuum graphite tube furnace. The chamber of the furnace, after evacuation, was filled with argon gas to a pressure of 60 torr, to prevent the rapid effusion of the experimental vapour from the open ends of the tube. The spectrum has been excited at about 2400°C and is recorded on Ilford HP-3 panchromatic plates. An exposure of six to ten minutes was found sufficient using Hilger E-492 large quartz spectrograph.

The  $C^2\Pi - X^2\Sigma$  system known in the spectral region  $\lambda\lambda 3990-4235$  has been extended on both the sides in thermal emission and was recorded to lie in the wavelength region  $\lambda\lambda 3919-4307$ . The bands were found to be double headed and clearly degraded to red. Their  $Q_2$  and  $Q_1$  heads can be well represented by the relation given below.

$$\begin{aligned} \nu_{01, Q_2} &= \frac{24343.68}{2465.81} + \frac{205.56}{204.92} (\nu'' - \frac{1}{2}) \\ &\quad - \frac{0.49}{0.48} (\nu'' - \frac{1}{2})^2 - \frac{216.62}{216.72} (\nu'' - \frac{1}{2}) \\ &\quad - \frac{0.51}{0.52} (\nu'' - \frac{1}{2})^2 \end{aligned}$$

Quite a good number of new bands including new sequences have been identified for this system.

TABLE I

New data and data for the systems C-X and D-X of the molecule—SrBr

| $\lambda$ in Å          | $\nu$ in $\text{cm}^{-1}$ |       | Analysis<br>( $\nu'$ , $\nu''$ ) |
|-------------------------|---------------------------|-------|----------------------------------|
|                         | Obs.                      | Calc. |                                  |
| 4019.8                  | 24870                     | 24866 | (9, 6)                           |
| 4021.8                  | 24857                     | 24852 | (10, 7)                          |
| 4081.5                  | 24494                     | 24495 | (5, 4)                           |
| 4113.4                  | 24304                     | 24305 | (3, 3)                           |
| 4115.4                  | 24292                     | 24294 | (4, 4)                           |
| 4177.3                  | 24289                     | 24283 | (5, 5)                           |
| 4183.3                  | 24772                     | 24073 | (5, 6)                           |
| 4185.4                  | 24051                     | 24063 | (6, 7)                           |
| 4186.7                  | 24051                     | 24053 | (7, 8)                           |
| 4181.5                  | 23909                     | 23908 | (0, 2)                           |
| 4198.2                  | 23813                     | 23811 | (11, 13)                         |
| 4200.1                  | 23802                     | 23803 | (12, 14)                         |
| 4210.9                  | 23685                     | 23686 | (1, 4)                           |
| 4222.5                  | 23676                     | 23679 | (2, 5)                           |
| 4231.0                  | 23462                     | 23461 | (3, 7)                           |
| 4262.3                  | 23455                     | 23454 | (4, 8)                           |
| 4263.4                  | 23449                     | 23447 | (5, 9)                           |
| 4264.4                  | 23443                     | 23441 | (6, 10)                          |
| 4265.5                  | 23476                     | 23434 | (7, 11)                          |
| 4300.8                  | 23245                     | 23241 | (5, 10)                          |
| 4302.9                  | 23234                     | 23230 | (7, 12)                          |
| 4307.1                  | 23211                     | 23207 | (11, 16)                         |
| System C-X, $Q_2$ heads |                           |       |                                  |
| 3915.5                  | 25530                     | 25524 | (14, 9)                          |
| 3918.6                  | 25512                     | 25508 | (15, 10)                         |
| 3936.6                  | 25395                     | 25393 | (9, 5)                           |
| 3939.0                  | 25380                     | 25378 | (10, 6)                          |
| 3941.3                  | 25366                     | 25363 | (11, 7)                          |
| 3960.9                  | 25240                     | 25240 | (5, 2)                           |
| 3963.3                  | 25224                     | 25225 | (6, 3)                           |
| 3965.5                  | 25210                     | 25211 | (7, 4)                           |
| 3967.4                  | 25198                     | 25197 | (8, 5)                           |
| 3969.6                  | 25184                     | 25183 | (9, 6)                           |
| 3988.5                  | 25065                     | 25067 | (2, 0)                           |
| 4027.4                  | 24823                     | 24826 | (4, 3)                           |
| 4029.3                  | 24811                     | 24814 | (5, 4)                           |
| 4031.3                  | 24799                     | 24802 | (6, 5)                           |
| 4125.4                  | 24233                     | 24230 | (0, 2)                           |
| 4164.6                  | 24005                     | 24004 | (1, 4)                           |
| 4166.4                  | 23995                     | 23999 | (2, 5)                           |
| 4168.1                  | 23985                     | 23990 | (3, 6)                           |
| 4181.7                  | 23907                     | 23902 | (14, 17)                         |
| 4186.4                  | 23880                     | 23873 | (18, 21)                         |
| System D-X, P heads     |                           |       |                                  |
| 3317.2                  | 30137                     | 30137 | (11, 7)                          |
| 3529.3                  | 28326                     | 28330 | (0, 3)                           |

Further on the shorter wavelength side a group of single headed bands was photographed in the spectral region  $\lambda\lambda 3317-3529$ . These bands belong to the system  $D^2\Sigma - X^2\Sigma$  and are degraded to shorter wavelength side. The bands for this system were found to be comparatively weak in intensity and

show a wide condon parabola. The wavenumbers of their P-heads can be given by the equation.

$$\nu = 28959 \cdot 17 - 247 \cdot 83 (\nu' - \frac{1}{2})^2 - 0 \cdot 55 (\nu'' - \frac{1}{2})^2 - 216 \cdot 62 (\nu' + \frac{1}{2}) + 0 \cdot 51 (\nu'' + \frac{1}{2})^2$$

A few new bands could also be recorded for this system. Table I represents the wavelengths of the new band heads with their corresponding wavenumbers in vacuum and vibrational analysis, etc., for the C-X and D-X systems.

The E-X system lying in the spectral region  $\lambda\lambda$  3000-3200 also appeared on the photographic plate but only faintly..

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## CRYSTAL STRUCTURES OF AMIDOPYRINE AND PHENYLBUTAZONE

As part of a programme of structural studies on analgesics and their interactions<sup>1-4</sup>, we have determined the crystal structures of two well-known analgesics, namely, 1-phenyl-2, 3-dimethyl-4-dimethyl-amino-5-pyrazolone (amidopyrine) and 4-butyl-1, 2-diphenylpyrazolidinedione (phenylbutazone), by x-ray diffraction methods. A preliminary account of these structure analyses is given here.

The space groups and unit cell dimensions of these compounds were reported earlier<sup>1</sup>. However, the cell dimensions of amidopyrine, though essentially correct, were found to be in considerable error and, hence, they were redetermined from oscillation and Weissenberg photographs and refined using high angle Bragg reflections. These cell dimensions and other relevant data along with those reported earlier for phenylbutazone are given below.

**Amidopyrine :** Space group  $P \bar{1}$

$$a = 7 \cdot 458 \pm 0 \cdot 005, \quad b = 10 \cdot 744 \pm 0 \cdot 005, \\ c = 17 \cdot 486 \pm 0 \cdot 015 \text{ \AA};$$

$$\alpha = 98 \cdot 6 \pm 0 \cdot 2, \quad \beta = 88 \cdot 6 \pm 0 \cdot 3, \quad \gamma = 108 \cdot 6 \pm 0 \cdot 2^\circ;$$

$Z = 4$ ;  $D_m = 1 \cdot 176 \pm 0 \cdot 005$ ,  $D_o = 1 \cdot 171 \text{ gm/cc}$ .  
**Phenylbutazone :** Space group  $P2_1/c$

$$a = 21 \cdot 695 \pm 0 \cdot 004, \quad b = 5 \cdot 823 \pm 0 \cdot 002, \\ c = 27 \cdot 881 \pm 0 \cdot 004 \text{ \AA};$$

$$\beta = 108 \cdot 06 \pm 0 \cdot 10^\circ; \quad D_m = 1 \cdot 211 \pm 0 \cdot 020, \\ D_o = 1 \cdot 218 \text{ gm/cc}, \\ Z = 8.$$

Three-dimensional x-ray data from the crystals of amidopyrine were recorded on multiple-film equi-inclination Weissenberg photographs using  $\text{CuK}_\alpha$  radiation for reciprocal levels  $hkl$ ,  $h = 0$  through 6, and  $hkl$ ,  $l = 0$  through 10, and the intensities were estimated visually. The intensity data from the crystals of phenylbutazone were collected on a 4-circle Hilger and Watts diffractometer at the Laboratory of Molecular Biophysics, Oxford. Both the structures were solved by direct methods using the programme MULTAN originally written by M. M. Woolfson, P. Main and G. Germain, and modified for the IBM 360/44 computer in the Institute by S. Ramkumar and M. R. N. Murthy. The solutions were aided by packing considerations and trial calculations. The structures were refined isotropically by the structure factor least squares method. The current R values are 0.16 for amidopyrine and 0.15 for phenylbutazone. The atomic positions at the present stage of refinement are shown in Figs. 1 and 2.

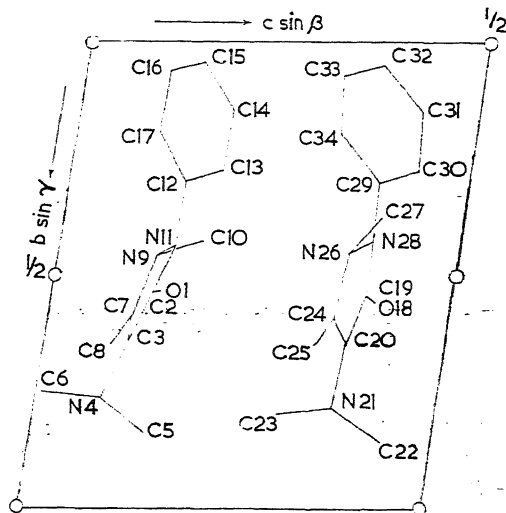


FIG. 1. The crystallographically independent molecules in the structure of amidopyrine as viewed along the  $a$ -axis.

Both the structures contain 12 molecules in the asymmetric part. In each structure, the crystallographically non-equivalent molecules have comparable bond lengths and angles. The dimensions of the pyrazolone ring in the amidopyrine molecule are broadly similar to those observed in the structure of antipyrine. The hetero nitrogen atoms in the ring are pyramidal. In the structure of phenylbutazone, the two C=O bonds in each molecule have lengths appropriate to a C=O double bond and, hence, neither of the oxygen atoms is protonated. The tetrahedral character of the carbon atom at the 4-position indicates that a hydrogen atom is attached to it. The two nitrogen atoms in the five-membered ring are pyramidal and the attached phenyl rings are *trans* about the N-N bond.

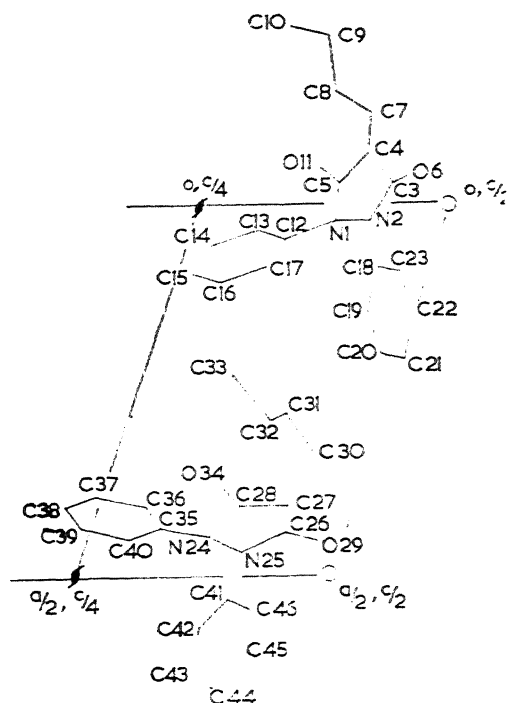


FIG. 2. The crystallographically independent molecules in the structure of phenylbutazone as viewed along the *b*-axis.

Further refinement of the structures is in progress. Details of the analyses and the final results will be published elsewhere.

The authors thank Professor G. N. Ramachandran and Professor V. S. Venkatasubramanian for their interest in this work and Professor Dorothy

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## EMISSION SPECTRUM OF PHENYLACETYLENE

THE emission spectrum of phenylacetylene has been studied although its ultraviolet absorption is reported by Padhye *et al.*<sup>1</sup> since we believe that generally the emission spectrum of a molecule yields more information on the ground electronic state than its absorption spectrum. The results obtained by us on the ultraviolet emission spectrum of phenylacetylene vapour are reported here.

The emission spectrum of phenylacetylene was excited by an uncondensed transformer discharge at about 1,500 volts through the flowing vapour of the substance in a  $\pi$ -type discharge tube of diameter 1.6 cm and length, 40 cm. The spectrum was photographed on a medium quartz spectrograph with an exposure of about 5 hours. The spectrum occurring in the region  $\lambda = 2715 \text{ \AA} - 3095 \text{ \AA}$  consisted of about 40 bands overlapped by a fairly strong continuum.

With the band at  $36369 \text{ cm}^{-1}$  chosen as the 0, 0 band which agrees well with the position ( $36,370 \text{ cm}^{-1}$ ) obtained in the absorption spectrum<sup>1</sup>, all the bands in the spectrum could be analysed in terms of 2 upper state and 13 ground state fundamental frequencies. Table I gives the band data and their assignments and also the nature of vibrations of the fundamental frequencies. Also in the parentheses are given in the same table, the Raman and infrared data<sup>2</sup> for comparison. In addition, frequencies, 60 and  $101 \text{ cm}^{-1}$ , have been observed as combination bands. These are probably due to  $v-v$  transition of some non-totally symmetric vibrations.

The symmetry of the molecule is represented approximately by the point group  $C_{2v}$  and the

TABLE I

*Assignment of the prominent emission bands in phenylacetylene*

| Wave number<br>( $\text{cm}^{-1}$ )<br>and intensity | Assignment                | Nature of vibration          |
|--|---------------------------|------------------------------|
| 36781 w  | 0, 0+ 412                 | Ring in-plane deformation    |
| 36580 ms   | 0, 0+ 211                 | C—C $\equiv$ C bending       |
| 36369 m  | 0, 0                      |                              |
| 36309 ms   | 0, 0— 60                  |                              |
| 36268 ms   | 0, 0— 101                 |                              |
| 36205 ms   | 0, 0— 164 ( 164R)         | C—X out-of-plane deformation |
| 36013 ms   | 0, 0— 356 ( 353R)         | C—C $\equiv$ C bending       |
| 35909 s  | 0, 0— 460 ( 465R)         | Ring in-plane deformation    |
| 35839 vs   | 0, 0— 530 ( 530R)         | C—C $\equiv$ C bending       |
| 35748 ms   | 0, 0— 621 ( 621R)         | Ring in-plane deformation    |
| 35604 w  | 0, 0— 764 ( 760R, 758IR)  | Ring breathing               |
| 35484 w  | 0, 0— 530—356             |                              |
| 35435 w  | 0, 0— 2 $\times$ 460      |                              |
| 35389 vs   | 0, 0— 980 ( 989IR)        | C—H in-plane deformation     |
| 35356 s  | 0, 0—1013 (1001R, 1002IR) | Ring in-plane deformation    |
| 35286 vs   | 0, 0—1083 (1072IR)        | C—H in-plane deformation     |
| 35162 ms   | 0, 0—1207 (1196R)         | C—X stretching               |
| 35094 w  | 0, 0—1207—60              |                              |
| 35049 w  | 0, 0— 980—356             |                              |
| 35990 w  | 0, 0— 764—621             |                              |
| 34891 ms   | 0, 0—1478 (1489R)         | C—C stretching               |
| 34794 ms   | 0, 0—1575 (1579R)         | do.                          |
| 34684 w  | 0, 0—1083—621             |                              |
| 34537 vw   | 0, 0— 621—1207            |                              |
| 34395 ms   | 0, 0—1207—764             |                              |
| 34259 ms   | 0, 0—2110 (2113R, IR)     | C $\equiv$ C stretching      |
| 34200 ms   | 0, 0—1083—621—460         |                              |
| 34079 w  | ..                        |                              |
| 34012 w  | 0, 0—1575—764             |                              |
| 33860 vw   | 0, 0—1478—1013            |                              |
| 33803 vw   | 0, 0—1573—1013            |                              |
| 33710 w  | 0, 0—1083—1575            |                              |
| 33655 w  | 0, 0—2110—621             |                              |
| 33534 vw   | ..                        |                              |
| 33439 w  | 9, 0— 2 $\times$ 1478     |                              |
| 33241 w  | 0, 0—1028—2110            |                              |
| 33175 w  | 0, 0—1083—2110            |                              |
| 33952 w  | 0, 0—2110—980—1356        |                              |
| 32618 vw   | ..                        |                              |
| 32427 vw   | 0, 0—1013—1478—1575       |                              |
| 32336 vw   | ..                        |                              |

vw=very weak, w=weak, m=medium, ms=medium strong, s=strong, vs=very strong.  
 IR=Infrared, R=Raman, X=substituent.

observed bands are ascribed to the electronic transition,  $A_1-B_1$  allowed for this molecule.

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### TITRIMETRIC DETERMINATION OF MILLIGRAM AMOUNTS OF COPPER

A GRAVIMETRIC procedure for the determination of copper as cuprous thiocyanate using ammonium thiocyanate both as reagent and precipitant was developed<sup>1</sup>. We have now evolved a titrimetric procedure by combining the gravimetric method mentioned and the Volhard's procedure<sup>2</sup> for the determination of copper. This new procedure permits determination of milligram amounts of copper.

**Procedure.**—A known volume of the standard copper sulphate solution was mixed with about 45 ml of glacial acetic acid and the contents were diluted to ca. 150 ml. The solution was then heated on a boiling water bath for about 30 minutes and a freshly prepared 10% ammonium thiocyanate solution was added drop by drop with constant stirring till the precipitate was just observed. About 5 ml of water were added and the heating continued till the supernatant liquid was clear. The contents of the beaker were kept at room temperature for 2 to 3 hours and the precipitate was filtered through a No. 4 sintered glass crucible. The precipitate was washed four to five times with cold 0.1% ammonium thiocyanate solution and finally with 20% ethanol till the precipitate was free from soluble thiocyanate. The precipitate was then dissolved in a requisite quantity (20–25 ml) of liquor ammonia. The solution collected in the buchner flask was quantitatively transferred into a 250-ml conical flask and mixed with nitric acid (6 N) until the cuprammonium blue disappeared followed by two to three ml in excess. A known excess of standard silver nitrate solution (0.05 N) was added. Ferric alum indicator (1 ml) was then added and the excess silver nitrate back-titrated with standard ammonium thiocyanate (0.05 N) solution.

TABLE I

*Volumetric determination of copper via precipitation with ammonium thiocyanate*

| Sl. No. | Copper, mg |        | Error % |
|---------|------------|--------|---------|
|         | Taken      | Found  |         |
| 1       | 5.610      | 5.595  | − 0.27  |
| 2       | 11.220     | 11.290 | − 0.62  |
| 3       | 16.830     | 16.920 | + 0.54  |
| 4       | 22.440     | 22.570 | + 0.58  |
| 5       | 28.050     | 28.130 | + 0.29  |

The results recorded in Table I clearly show that milligram amounts of copper could satisfactorily be determined by this method. Moreover, this titrimetric procedure could be extended for the determination of copper in ores and alloys, since copper could be precipitated as cuprous thiocyanate in presence of several other metals.

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### SPECTROPHOTOMETRIC STUDY OF SCANDIUM CHELATE WITH 2, 2'-(ETHANE-DIYLIDENEDINITRILE); DIPHENOL (GBHA)

The Schiff base GBHA is prepared by the reaction of glyoxal and O-aminophenol<sup>1</sup>. The GBHA is colourless but its chelates with metal ions are intense violet to red. The analytical applications of GBHA have been surveyed by Jungreis and Thaber<sup>2</sup>. Vrchlabsky and Bocek<sup>3</sup> have used GBHA for the determination of Sc (III) in aqueous solution. In the present work scandium (III) complex with GBHA has been studied by extraction in *n*-butanol in presence of acetate and perchlorate ion. In this procedure wavelength for maximum absorbance remains same but there is an eight-fold increase in intensity.

A Unicam spectrophotometer, SP 600 and a Metrohm pH-meter, E-350 were used for measuring the absorbance and pH's of the solutions. Solution of the ligand of appropriate strength was prepared in *n*-butanol. Scandium (III) solution was prepared by dissolving Sc<sub>2</sub>O<sub>3</sub> (G.R. Merck) in perchloric acid and solution made up to the mark with double distilled water. All other chemicals used were of reagent grade.

**Recommended procedure.**—To suitable aliquots of Sc (III) add excess GBHA in *n*-butanol in presence of acetate and perchlorate ions at pH 5–10. The solutions are then shaken for about two hours. The two layers are separated and absorbance of the complex is then measured against the corresponding reagent blank.

**Absorption spectra of the complex.**—Solutions containing Sc (III) and GBHA in ratio 1 : 50 were taken in the pH range 4.0 to 7.0. The complex extracted with *n*-butanol and spectra were recorded against the reagent blanks. The complex found to exhibit maximum absorbance at 560 nm in the pH,

range 4.80 to 5.30. Beyond this pH, the absorbance falls sharply.

**Stability of the complex.**—The reddish violet colour of the complex is extracted completely in *n*-butanol in two hours and colour intensity was found to be stable for 42 hours at  $25 \pm 1^\circ$ .

**Characteristics of the complex.**—The molar composition of the complex as deduced by Asmus<sup>4</sup> and Bent and French<sup>5</sup> was found to be 1:1. The system obeys Beer's law up to 3.8 ppm of scandium (III) at pH 5.0 and at  $\lambda = 560$  nm. The sensitivity of the colour reaction is  $0.0044 \mu\text{g Sc/cm}^2$  for  $\log I_0/I = 0.001$ . The optimum range for accurate determination as obtained from Ringbom plot<sup>6</sup> is 1.2 to 3.0 ppm.

**Discussion.**—Vrchlabsky and Bocek<sup>3</sup> have studied Sc (III)—GBHA system and reported that when scandium (III) solution is heated with an aqueous ethanolic solution of GBHA in presence of  $\text{NH}_4\text{NO}_3$  at pH 2.5 to 5.0, it forms a reddish complex which has an absorption maximum at 560 nm and sensitivity is  $0.033 \mu\text{g/cm}^2$ . They have also reported that acetate masks the reaction.

In the present work the species formed is extracted in *n*-butanol readily. No heating is required and the acetate ions do not interfere. The sensitivity is about 8 times more as compared to the sensitivity in aqueous medium. The 1:1 complex of scandium and GBHA is stable up to 42 hours whereas the species in aqueous solution is stable for a much shorter period.

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# SPECTROPHOTOMETRIC DETERMINATION OF OSMIUM WITH $\beta$ -BENZOYL- $\alpha$ -(*o*-TOLYL) THIOUREA

THE organic reagent, thiourea and its derivatives have been used for the spectrophotometric determination of osmium<sup>1-3</sup>. The present author has introduced benzoylthioureas, as sensitive and highly selective reagents for the same element<sup>4,5</sup>. In this communication, is described the use of one such reagent,  $\beta$ -benzoyl- $\alpha$ -(*o*-tolyl) thiourea, for the

spectrophotometric determination of osmium. The orange-yellow complex of osmium formed with the reagent is soluble in ethanol and in a number of other organic solvents. In solvents like ethanol and chloroform the complex obeys Beer's law over a useful range of concentration of osmium and microgram amounts of the element can be determined in presence of a large number of foreign ions. Besides the analytical aspects, studies have also been made with regard to the composition and dissociation constant of the complex in solution utilising spectrophotometric methods.

The reagent was prepared by the method reported earlier<sup>1</sup>. A 0.5% solution of the reagent in ethanol was used. A standard osmium (VIII) solution was prepared as reported in an earlier communication<sup>6</sup>. A Unicam SP 600 spectrophotometer was used for absorbance measurements. All other solutions were prepared from reagent grade chemicals.

**Procedure.**—A standard aliquot of osmium (VIII) solution was treated with 3 ml of 0.5% ethanolic solution of the reagent. The acidity of the solution was adjusted to 6 N with conc. hydrochloric acid. After the addition of 5 ml of ethanol, the solution was heated for ten minutes over a boiling water bath. The mixture was cooled and made up to 25 ml with ethanol. The solution can also be extracted with chloroform and the extract diluted to 25 ml with the same solvent. The absorbances of the solution thus obtained was measured against a blank prepared similarly.

**Results and Discussion.**—The osmium complex was found to be soluble in ethanol, chloroform, carbon tetrachloride, ethyl acetate, amyl acetate, amyl alcohol and tri-*n*-butylphosphate. In ethanol and chloroform the complex shows maximum absorption at 400 nm and all the measurements were carried out at this wavelength. In both these solvents Beer's law is obeyed from 3 to 21 ppm of osmium and the Ringbom plot<sup>7</sup> shows the optimum range of 6 to 18 ppm. The maximum colour intensity is observed in 4–8 N HCl and 2 ml (or more) of the reagent solution was necessary for full development of colour. The Sandell sensitivity<sup>8</sup> of the colour system was found to be  $0.028 \mu\text{g/cm}^2$  and the per cent. relative error<sup>9</sup> was 2.72%. The molar absorptivity was 6800.

The composition of the complex in solution was ascertained by the Job's method<sup>10</sup> and the molar ratio method<sup>11</sup>. Both the methods indicated the formation of a 1:1 complex.

The dissociation constant was evaluated from the Job's method of nonequimolar solutions<sup>12</sup> and the method of Harvey and Manning<sup>13</sup>. The dissociation constant, *K*, was  $8.1 \times 10^{-4}$  by the Job's

method. K<sub>2</sub> as calculated from the Harvey and Manning equation was 3.0 × 10<sup>-4</sup>.

With the help of this equation the value as 6 ppm of osmium VIII can be determined after extraction of the complex into chloroform in presence of 4.0 ppm of the ions of Al<sup>3+</sup>, Ti<sup>4+</sup>, Fe<sup>3+</sup>, Mn<sup>2+</sup>, Ba<sup>2+</sup>, Na<sup>+</sup>, UO<sub>2</sub><sup>2+</sup>, As<sup>3+</sup>, Hg<sup>2+</sup>, ZrO<sub>2</sub><sup>2+</sup>, V<sup>5+</sup>, Mg<sup>2+</sup>, Cd<sup>2+</sup>, Cu<sup>2+</sup>, W<sup>6+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Sr<sup>2+</sup>, Bi<sup>3+</sup>, Co<sup>2+</sup>, Cr<sup>3+</sup>, Zn<sup>2+</sup>, La<sup>3+</sup>, Th<sup>4+</sup>, Sb<sup>3+</sup>, Pb<sup>2+</sup>, chromate, manganate, oxalate, phosphate and the rare earths, and 100 ppm of In<sup>3+</sup> and Pd<sup>2+</sup> and 40 ppm of Rh<sup>3+</sup> and Pt<sup>4+</sup>. Interference due to palladium was avoided by prior removal as its azide complex. However, the ions of Cu<sup>2+</sup>, Ag<sup>+</sup>, Au<sup>3+</sup> and Ru<sup>3+</sup> interfere with the determination.

The author wishes to express his deep sense of gratitude and indebtedness to Prof. Dr. A. K. Majumdar, Senior Professor of Chemistry, Jadavpur University, Calcutta-32, for his keen interest and valuable suggestions.

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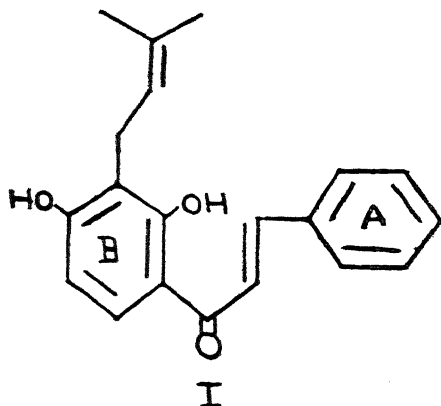
# FLEMISTRICTIN-A, A NEW CHALCONE FROM THE LEAVES OF *FLEMINGIA STRICTA* ROXB. (LEGUMINOSAE)

IN continuation of our work on the chemical examination of the plants belonging to the genus *Flemingia* (Leguminosae), we have examined the leaves of *F. stricta* Roxb. from the hexane extract of which a new prenylated chalcone designated as flemistricitin-A has been isolated.

The hexane extract of the dried powdered leaves on concentration gave a greenish yellow residue

which was subjected to column chromatography on silica gel (20 g) with benzene : hexane (3 : 1) and eluted successively with benzene : hexane (3 : 1), benzene and benzene (fraction-2). The later fractions of benzene : hexane (3 : 1) eluate on concentration gave waxy material. Most of the leaves were removed by crystallisation from methanol and the residue from the filtrate on crystallisation from hexane gave orange-yellow needles of flemistricitin-A.

Flemistricitin-A melted at 164° (Found C 77.91, H 6.28, C<sub>20</sub>H<sub>20</sub>O<sub>2</sub> requires C 78.02, H 6.49%). M<sup>+</sup> 308. It gave characteristic test for chalcones with SnCl<sub>4</sub> in CCl<sub>4</sub>.  $\lambda_{\text{max}}^{\text{EtOH}}$  215 and 356 nm.  $\mu_{\text{max}}^{\text{NaCl}}$  3150 (hydroxyl), 1620 (chelated carbonyl) and 1370 cm<sup>-1</sup> (gem-dimethyl group). The 60 MHz NMR spectrum of flemistricitin-A in CHCl<sub>3</sub> showed an AB quartet (7.93, 7.50 J<sub>trans</sub> = 16 Hz) and a chelated hydroxyl at 14.33 (singlet, disappeared on D<sub>2</sub>O exchange) providing evidence for the chalcone system. A doublet at 3.50 (2H, J = 7 Hz) for an Ar-CH<sub>2</sub>-, a vinylic hydrogen adjacent to a CH<sub>2</sub> at 5.30 (1H, triplet) and two singlets at 1.80 (3H) and 1.87 (3H) for two methyl groups on olefinic carbon atom showed the presence of a 3,3-dimethylallyl group attached to an aromatic ring. The two ortho-coupled protons on ring B appeared at 6.45 (1H, doublet, J = 9 Hz) and 7.30 (1H, doublet, J = 9 Hz) and the five aromatic protons on ring A appeared as a multiplet between 7.10–7.40. Mass spectrum of flemistricitin-A showed a molecular ion m/e 308 and other fragment ions at m/e 293 (M-15), m/e 275 (M-18-15), m/e 265 (M-43), i.e., (M-C<sub>3</sub>H<sub>7</sub>), m/e 252 (M-56), i.e., (M-C<sub>4</sub>H<sub>8</sub>), m/e 231 (M-77), m/e 204 (M-104), m/e 175 (M-77-56) and m/e 148 (M-104-56).



The peaks at 104 and 77 clearly indicated the unsubstituted nature of ring A. From the above data flemistricitin-A has been assigned structure I.

The structure of hemistriclin-A has been confirmed by its synthesis.  $\beta$ -Resacetophenone was prenylated<sup>1</sup> using methanolic KOH and the 3-C-prenyl derivative was subjected to chalcone condensation with benzaldehyde. The product was purified by column chromatography over silica gel using benzene as eluant. The compound crystallised as orange-yellow needles which was identical with the natural compound in all respects (m.p., m.m.p., UV, IR, and NMR).

The benzene eluate (fraction-2) on concentration and crystallisation from methanol gave colourless crystals, m.p. 138°; identified as  $\beta$ -sitosterol by direct comparison with an authentic sample (m.m.p. and co-TLC).

We are thankful to Dr. M. Ananthaswamy Rao, Deputy Director, Botanical Survey of India, Dehra Dun, and Dr. P. S. Prakasa Rao, Department of Botany, A.U.P.G. Centre, Guntur, for the supply of plant material and identification. One of us (J. M. R.) thanks C.S.I.R. for the award of Research Fellowship.

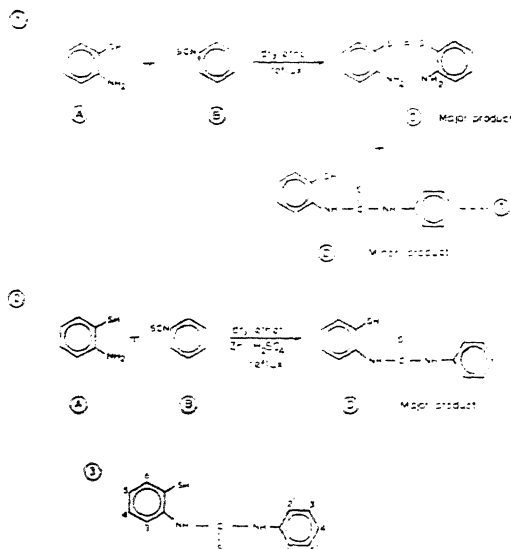
Dep't. of Chemistry, J. MADHUSUDHANA RAO.  
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# SYNTHESIS OF N-(1-MERCAPTOPHENYL)- N'-PHENYLTHIOUREA AS A CHELATING AGENT

THE preparation of N-hydroxy-N'-diaryl thioureas and N-(1-hydroxyphenyl)-N'-phenyl thiourea and their use as chelating agents have been reported in the literature<sup>1</sup>. The latter compound was obtained by condensing o-aminophenol with phenylisothiocyanate in refluxing dry ether. We found that under identical experimental conditions, condensation of o-aminobenzenethiol with phenyl-isothiocyanate was difficult. The yield of the condensation product was very poor, the dimerized compound (C) being the major product. However when mild reducing conditions were employed, the yield of the desired product (D) could be increased considerably.

When a pinch of zinc dust and about 0.1 ml. of concentrated sulphuric acid were added to the ether solution of (A) and (B) and the solution refluxed for three hours, (D) was the major product of the reaction. The yield of (D) could still be increased, first by dimerizing (A) to yield (C), and then condensing (C) with (B) and finally reducing the condensed product with Zn dust and sulphuric acid.



The compound (D) is readily susceptible to air-oxidation, hence it was stored in sealed tubes, and all the reactions carried out in an inert atmosphere of nitrogen. The n.m.r. spectrum of (D) taken in CDCl<sub>3</sub> shows a broad signal around 4.5  $\delta$  accounting for three protons (2NH, one SH). The aromatic region between 6.5-7.2  $\delta$  corresponds to 5 shielded aromatic protons which may be due to hydrogens at 2', 6', 3, 6, and 4 (or 5), while a strong signal appears around 7.3-7.5  $\delta$  which can be assigned to those at 3', 4', 5' and 5 (or 4).

The compound (D) readily forms complexes with bivalent transition metals like Cu<sup>+2</sup>, Ni<sup>+2</sup>, Co<sup>+2</sup>, etc. The elemental analysis given in Table I

TABLE I  
Elemental analysis

| Compound   | Elemental Analysis |       |       |       |       |       |         |       |
|------------|--------------------|-------|-------|-------|-------|-------|---------|-------|
|            | % C                |       | % N   |       | % S   |       | % Metal |       |
|            | Cal.               | Found | Cal.  | Found | Cal.  | Found | Cal.    | Found |
| Ligand     | 60                 | 59.85 | 10.76 | 10.6  | 24.61 | 24.7  |         |       |
| Cu-complex | 53.65              | 53.5  | 9.62  | 9.42  | 23.7  | 23.5  | 10.91   | 10.81 |
| Ni-complex | 54.11              | 54.00 | 9.71  | 9.62  | 23.93 | 23.8  | 10.18   | 9.95  |
| Co-complex | 54.1               | 54.1  | 9.69  | 9.62  | 23.9  | 23.83 | 10.17   | 9.87  |

clearly indicates that the Metal : Ligand ratio in the complexes is 1:2 and their infrared spectra data strongly suggest that their structures are analogous to the complexes of the same metals with  $N$ -(1-hydroxyphenyl)- $N'$ -phenyl thiourea<sup>1</sup>.

**A. Synthesis of the Ligand—*p*-aminobenzensulpho-**  
13 g. and phenyl isothiocyanate (14 g.) were added to a mixture of 25 ml. of dry ether and 0.5 ml. of concentrated  $H_2SO_4$ . Zn dust was then added to this mixture and refluxed under nitrogen for three hours. On cooling, a solid separated out. After removing the ether, the solid was extracted with 2N sodium hydroxide. The solution was filtered and the compound was regenerated from the filtrate by dropwise addition of glacial acetic acid with constant stirring. The solid was filtered and recrystallised from ethanol.

**B. Synthesis of complexes.**—Equimolar alcoholic solutions of the ligand and metal-nitrates ( $Cu^{++}$ ,  $Ni^{++}$ ,  $Co^{++}$ ) were mixed separately and kept in deep freeze overnight. The precipitates obtained were removed by filtration and washed with alcohol to remove the excess of metal-nitrates. The resulting complexes were recrystallised from alcohol.

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## EVIDENCE OF RELICT SALINITY AND MARINE TRANSGRESSION IN THE SHALLOW SOUTH- WESTERN FRINGE OF THE NARMADA UPLAND ALLUVIAL VALLEY OF MADHYA PRADESH

OCCURRENCE of the deteriorating quality of water from the Timurni Block of Seoni Malwa Region of Madhya Pradesh was first recorded by A. S. M. Rao (1961) from the well at Pokharni (22° 25' 77" 13'). To delineate the pockets of salinity, seven samples from the fringe belt were subjected to chemical analysis. Electrical conductivity of these samples varies from 960 to 6,750  $\mu$ mhos/cm at 25°C, suggesting a sudden deterioration in quality towards the fringe belt of the region. There are indeed six such isolated pockets in the area, namely, Rala, Tajpura-Pokharni-Timurni, Gurari, Dhanpura, Sirkhamba and Bariaon. The high values of electrical conductivity are further supported by the sympathetic values of the chloride ions, and the abnormally high value of 1,470 ppm of chlorides at Naosar. Further, it has been observed in the case of Pagdhal (22° 25' 77" 21') borehole, in the same area, that the salinity increases

with depth. In the diamond-shaped Piper diagram all the water samples fall essentially in areas 6 and 7, indicating noncarbonate hardness and noncarbonate alkali (Primary Salinity) respectively. All the water samples are thus in conformity with the mines and ocean waters. In the Collins bar diagram, the ratio of  $Cl$  to  $CO_3 + HCO_3$  varies from 1.5 to 26, suggesting again a rapidly deteriorating quality of water. Indeed water sample from Bariaon in the south-western trappean fringe belt has reached a very high ratio of 26 to confirm the relict salinity of the highest order matching with the sea-shore salinity, and thereby confirming also the evidence of marine transgression in the area and the link of the erstwhile channel of the Narmada with that of the Tapi-Purna.

Hence the marine transgression of the Eocene times has not only connected the Arabian Sea with the Purna valley through the arm of the Tapi valley (Adyalkar, 1963, p. 37) but also provides an access to the Narmada valley, and that the river Tapi served as the sole channel outlet for the entire Tapi-Purna-Narmada river system of post-Eocene times. Similar study of quality deterioration leading to the marine transgression was earlier demonstrated by Adyalkar *et al.* (1969) to explain the marine transgression of Middle Jurassic epoch along the Lathi-Jaisalmer contact in Jaisalmer District of Rajasthan.

This also brings home the fact that the Taylor's Sixth Groundwater Province (Taylor, 1959, p. 695) comprising Cainozoic Fault Basins of Peninsular India was a more composite unit with two major rivers of Narmada and Tapi having a remarkable westerly drainage. It was indeed a unique lake region of the Eocene times with a single channel of the Tapi serving as an outlet for both the tributaries, the Purna and the Narmada, flowing over the silted lake basins until the Pleistocene times, when the present topography came into existence with change in the course of the river Narmada to its present channel.

Central Ground Water Board, P. G. ADYALKAR,  
Nagpur, August 9, 1974. K. R. SRINIVASAN,  
M. A. HASEEB.

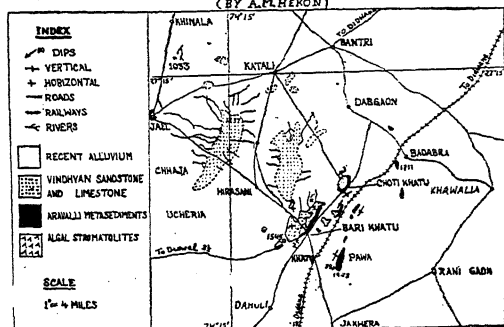
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# PHOSPHATIC ALGAL STROMATOLITES IN THE TRANS ARAVALLI VINDHYANS OF BADI KHATU, DISTRICT NAGOUR, RAJASTHAN

A NEW horizon of Algal Stromatolites has been discovered in the Trans Aravalli Vindhyan limestone around Badi Khatau (27° 7' : 74° 19'). District Nagaur, Rajasthan. The limestone with the algal structures occupies the top position in the sequence of Vindhyan rocks, i.e., on the top of the flat topped hills of Vindhyan rocks, resting unconformably over the Aravalli meta sediments in the plains of recent alluvium. Here the Vindhyan rocks have a gentle dip (about 10°) towards North-West with the North-East South-West strike, whereas the Aravalli rocks are folded and refolded. The lithology and the texture of the Vindhyan rocks here indicate the marine littoral environment of deposition, not very far off from the source where the continuous wave action resulted in good sorting of the underlying sandstone with angular grains. The underlying sandstone also shows sedimentary structures like bedding, cross-bedding, graded-bedding, ripple marks and other shallow water surface-structures. The geology of the region is well established through the work of Heron (1953), Krishnan and Swaminath (1959), Roy Choudhary *et al.* (1965) and Misra (1969).

Here, the uppermost rock unit of Vindhyan, on which the algal structures have grown in ferruginous and conglomeratic calcareous rock with slight angular fragments of quartz, agate, chert and limestone bounded by the ferruginous lime mud.

## GEOLOGICAL MAP OF KHATU AREA (BY A. P. HERON)



One very important aspect about the stromatolites of this area is that they have grown upto only a few inches in height. This phenomenon might have taken place due to the check in the growth of the algal stromatolites caused by the upheaval of the basement or change in the climatic conditions favourable for their growth. No doubt the later weathering has also played an important role in reducing the original thickness of the stromatolitic horizon.

The algal stromatolitic assemblage observed in this area includes : Weedia, Conophyton, Collenia-Columbaria, Fenton and Fenton, Collonia Baicalica-Maslov, Collonia Kuisensis-Maslov, Collonia Symmetrica, Krylov and Minjaria Calceolata-Korolyuk Minjaria Uralica. The characters of the individual species are similar to those described by Valdiya (1963) and Benerjee (1971). Mostly the stromatolite species are phosphatic in nature.

In a number of forms the black shining colophane is commonly coated on both sides of the individual laminae and sometimes it completely surrounds the algal structure. Mineral dehlite is also quite common. At the base of the algal structures the fragments of quartz, agate, chert and limestone are generally present. In some forms lime mud together with rock fragments has also occupied the intercolumnar spaces of the algal structures. Iron in form of hematite and limonite has completely altered the horizon containing the algal structures. The area openly invites the extensive studies.

Author expresses his gratitude to Mrs. S. Paliwal for her enthusiastic assistance. Help received from colleagues Mr. Khandelwal, Mr. Khan and Mr. Chauhan is also gratefully acknowledged.

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## ON THE SQUAT LOBSTER, *THENUS ORIENTALIS* (LUND) OFF VISAKHAPATNAM (BAY OF BENGAL)

*Panulirus polyphagus* (Herbst), *Panulirus ornatus* var, *Panulirus dasyptus* (H. Milne Edwards) and the deep sea *Puerulus sewelli* are common species of spiny lobsters and *Thenus orientalis* (Lund) and *Scyllarus sordidus* are common among the squat lobsters<sup>1</sup>.

*Tetralia orientalis* is common in the catches and is available more or less throughout the year with the peak during December to March, the breeding season of the animal. *Tetralia orientalis* and the related form, namely *Scyllarus solidus* are comparatively rare in the catches along the Bombay coast (Chhapgar and Deshmukh).

Berried females are available from December to July with a peak during winter season.

Some characters of *T. orientalis* from Visakhapatnam: body depressed; carapace broader than long; stalked eyes at the outer angles of the carapace; carapace and abdomen with granules or tubercles; abdominal segments with median ridge. 5th abdominal segment with three spines on each side and median comparatively bigger spine. Colour of the animal muddy grey. Dimensions of the animal are as follows:

| Sex         | Total length in mm | Carapace length in mm | Carapace breadth in mm | Total weight in gm |
|-------------|--------------------|-----------------------|------------------------|--------------------|
| ♀ (berried) | 248                | 85                    | 99                     | 269.8              |
| ♂           | 234                | 79                    | 94                     | 254.0              |

The estimated total number of eggs of the berried female is 12,467 with an average diameter of 1.1204 mm. Berried specimens are also found with mature and maturing ova, indicating that the species might spawn more than once in the same year.

*T. orientalis* had been recorded from India by White (1847), Neuman (1865), Orman (1891), Thompson (1901), from Madras by Heller (1865) and Henderson (1893), from Mundapam by Prasad and Tampi (1957), from Orissa coast by Alcock (1902) and from Bombay coast by Chhapgar and Deshmukh (1964). Prasad and Tampi<sup>2</sup> and Chhapgar and Deshmukh<sup>1</sup> have suggested that the winter is the breeding period, although Prasad and Tampi<sup>2</sup> mentioned a case of berried female caught in July and the larvae hatched in the same month. The world distribution ranges from Mauritius, Red Sea, Persian Gulf, East Indies and Australia to China.

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## ON A RARE BRACHYURAN CRAB ZOEA FROM THE SOUTH-WEST COAST OF INDIA

While sorting out the brachyuran larvae from the plankton samples collected by the U.N.D.P. Pelagic Fisheries Project from the south-west coast of India, an interesting brachyuran zoea with four lateral carapace spines was obtained from a sample from a station off Kasargod covered by the vessel 'Sardineilla' on 6-1-1972. Records of zoea having four lateral carapace spines are very few and such a larva has not so far been reported from the Indian waters.

Length of the zoea from the tip of the rostrum to the tip of the dorsal spine is 6.3 mm. The larva is characterised by the presence of a dorsal, rostral and four lateral carapace spines with small sharp spinules on its surface. All the four lateral spines (Fig. 1) show small enlargements at their extremities. In the abdomen, the 6th segment is separated

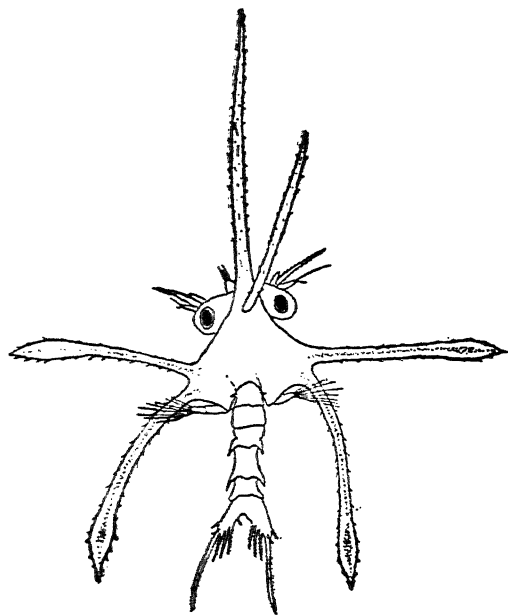


FIG. 1. 3rd zoea of *Tetralia* sp.

from the telson. The second abdominal segment has lateral spinular process on either side midlaterally. segment 3 is without any spine, while the 4th and 5th segments have each a prominent spine postero-laterally. Forked telson with long and slender arms bears 3 + 3 inner plumose spines. Of the two lateral spines on the sides one is dorsal in position. Posterior to the 3 inner spines, a small spine is seen only on one side. A small stump seen under high magnification in its place on the other side suggests that it might have been broken and lost. The presence of rudimentary buds of the

pleopods and the development of other appendages indicate that it is in the early 3rd zoeal stage.

The specimen with pairs of lateral spines on the carapace resembles the zoea of the xanthid crab *Tetralia glaberrima* (Herbst) described by Gurney<sup>1</sup> and Al-Kholy<sup>2</sup>, from the Red Sea. But an important difference noticed in the present specimen from their descriptions and figures is that the lateral spines are expanded at the tips. Such a zoea with two pairs of lateral spines having expanded tips has been figured by Dohrn<sup>3</sup>. Williamson<sup>4</sup> has also figured an unknown larva (Fig. 521) from the Atlantic with the same character of expanded extremities of lateral spines. But neither the lateral processes on the abdominal somite 2 nor the lateral spines on the telson found in the present specimen, as well as in the larva of *Tetralia glaberrima*, are seen in the figure by Williamson<sup>4</sup>. However, it could be said with certainty that the present zoea belongs to the genus *Tetralia*.

Most of the characters of the zoea agree well with those of the 3rd zoea stage of *Tetralia glaberrima*. But the important differences noticed are the expansion of the tips of the lateral carapace spines and the larger size of the larva. Along with these the absence of any spine on the 3rd abdominal somite makes it difficult to say that this is the larva of *Tetralia glaberrima* which is the only species of the genus recorded from the Indian waters<sup>5</sup>. Perhaps the larva belongs to some other species of *Tetralia* as yet not recorded from these waters.

Regional Centre,  
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## STUDIES ON *GRAPHIOLA PHOENICIS* (MOUG.) POIT.

### I. Histopathological Effects

*Graphiola phoenicis* (Moug.) Poit., the common false smut, occurs in the form of cup-shaped fruiting bodies on both the surfaces of pinnae, all round the rachis and on the leaf bases of *Phoenix sylvestris* Roxb. It is widely distributed in India,

Algeria, Egypt, Italy, Austria, Germany, Holland, Belgium, England and United States (Smith<sup>1</sup>). Except the work of Fischer<sup>2</sup> and Sharma<sup>3</sup> on the morphology, and Millard<sup>4</sup> on the cytology of this systemically controversial fungus, much work has not been done on other aspects, and practically the genus has been completely overlooked for its histopathological effects on the host.

The pathogen occurs on the leaves of *Phoenix sylvestris* in the form of black spots having the cup-like structures called "fruiting bodies". The latter consists of black, circular peridium surrounding the internal light yellow coloured sporogenous filaments and fascicles of sterile hyphae protruding out from the cup.

The mycelium is well developed, intercellular or sometimes intracellular, septate and branched. It forms a compact felt-like mass below the epidermis, from where arise sterile as well as fertile hyphae.

Considerable effect is produced on the various tissues of the lamina of infected host. The infection starts from the periphery and gradually spreads towards the centre of lamina.

Epidermal cells in the normal uninfected lamina are oblong or globose in shape, measuring  $3.99 \mu$  in length and  $2.66 \mu$  in breadth. After the development of the fruiting bodies, the epidermis is destroyed and sloughs off. The mycelium accumulates below the epidermis that gradually causes to raise the epidermal cells. They are later on distorted and ruptured in fragments at places to create spaces for developing mycelium that forms the fruiting body. Below the fruiting body, even remnants of epidermal cells are not present. Epidermal cells, adjoining the fruiting bodies become oval or rounded, are hypertrophied, and measure upto  $9.31 \mu$  in length and  $6.65 \mu$  in breadth. In some cells, the protoplasm shrinks and migrates towards the periphery (Fig. 1).

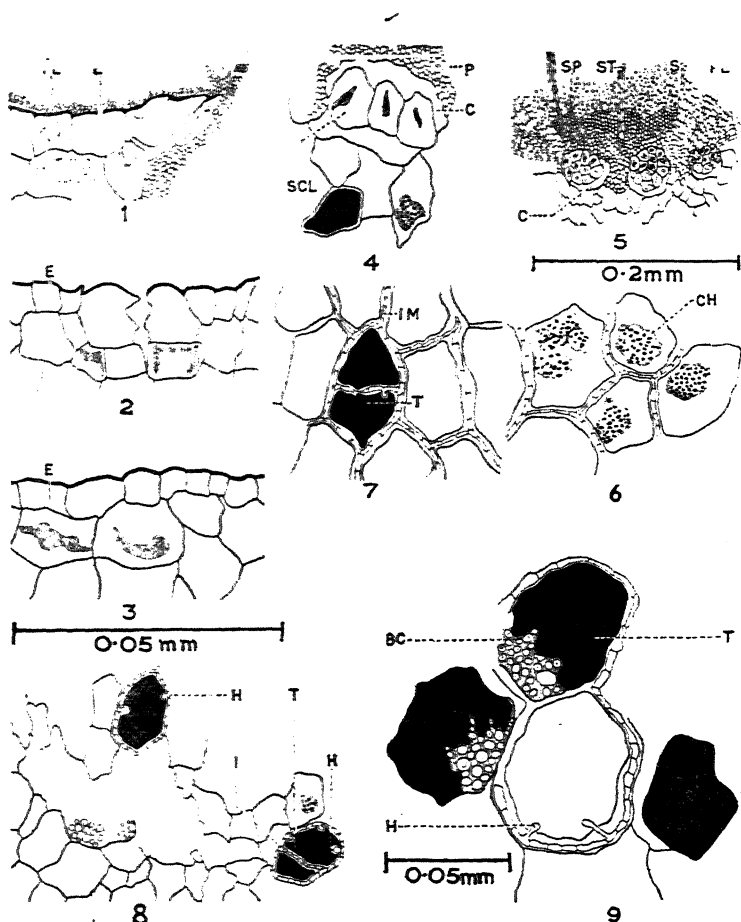
Hypodermal cells in the uninfected regions are cubical or slightly longitudinally extended and measure from  $6.65 \mu$  to  $10 \mu$  in length and from  $7 \mu$  to  $10 \mu$  in breadth. But they are hypertrophied below very young fruiting bodies and measure upto  $12 \mu$  in length and  $13.30 \mu$  in breadth. In places where the young fruiting bodies are developing, involving the hypodermal cells directly, many of these cells become disintegrated and disfigured and may be represented only by conical or irregular appearances. Hypodermal cells below well-developed fruiting bodies are ultimately destroyed by the pathogen and the spaces created are covered over by the fungus mycelium. In some of the hypodermal cells of the region adjoining the fruiting bodies, the cell contents contract and become rod-shaped, 'H'-shaped (Fig. 2) or coma-shaped (Fig. 3).



Sclerenchyma of the lamina is infected comparatively to a lesser extent. There is no significant change in these cells below very young fruiting bodies. But in severe infections, the complete sclerenchyma patch is pushed aside leaving a cavity (Figs. 4 and 5). Size of the normal sclerenchymatous cells measures  $7.98\mu$  in length and  $6.65\mu$  in breadth and  $26.60\mu$  in length while the normal uninfected cells measure from  $6$  to  $13\mu$  in breadth and upto  $20\mu$  in length. Because of the high degree of infection, separation of some groups of host cells takes place, forming many spaces in the ground tissue (Fig. 8).

Ground tissue of lamina is the badly affected region. The mycelium traverses in the narrow

intercellular spaces of the chlorenchymatous cells. In the early stages of the fungal infection, some of the cells of the ground tissue, which are in direct contact with pathogen, gradually become disintegrated. The cells, adjacent to the infected region, show hypertrophy, measuring upto  $23.94\mu$  in breadth and upto  $20\mu$  in length. Because of the high degree of infection, separation of some groups of host cells takes place, forming many spaces in the ground tissue (Fig. 8).



FIGS. 1-9. Figs. 1, 2 and 3. Epidermal and hypodermal cells in transverse section showing contraction of protoplasm. Fig. 4. Sclerenchymatous patch showing the formation of cavity. Fig. 5. Basal part of a well-developed fruiting body. Fig. 6. Cells of the chlorenchyma in transverse section. Fig. 7. Cells surrounded by intercellular as well as intracellular mycelium. Fig. 8. Formation of the space in the ground tissue. Fig. 9. Showing disorganization of the tannin filled cells.

BC, Burst cell; C, Cavity; CH, Chloroplasts; E, Epidermis; H, Haustorium; I, Intracellular mycelium; IM, Intercellular mycelium; P, Pseudoparenchymatous mass of mycelium; PE, Peridium; S, Spores; SCL, Sclerenchyma; SP, Sporogenous hyphae; ST, Sterile bundle; T, Tannin filled cell.

Due to the infection, the tannin-filled cells contract and thus become irregular in shape. Some of these cells burst off when the infection is high (Fig. 9). Inter as well as intracellular mycelium with knob-like haustoria is also seen around these cells (Figs. 7 and 8).

Chloroplasts are also infected at early and late stages of the fungus infection. They occupy peripheral position (Fig. 6). Sometimes, the cells are observed to be devoid of chloroplasts. The portion of the lamina thus becomes chlorotic and starts drying.

No well-marked effect has been observed on different tissues of vascular bundles. Only some cells of the bundle sheath show hypertrophy.

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# GAMETIC STERILITY INDUCED BY GAMMA RAYS IN *LUFFA ACUTANGULA* ROXB.

The intention of the present experiment was to record the amount of sterility as contributed by either the male or the female gamete, or by both, in *M<sub>1</sub>* plants of *Luffa acutangula*, a vegetable plant,

grown from dry seeds irradiated with gamma rays at 20, 30 and 40 kR dose levels.

The pollen grains were stained in Belling's acetocarmine solution and were scored for viability (Table I). In control 94% viability was observed. Results showed an inverse relationship between pollen viability and dose level and this might be due to genetic damage<sup>1</sup> and/or physiological disturbance<sup>2</sup>. Selfing and crossing operations between treated and untreated plants were classified under four categories, viz., (A) control selfed, (A) *M<sub>1</sub>* × control, (C) control × *M<sub>1</sub>* and (D) *M<sub>1</sub>* selfed. Each category was repeated ten times and then the mean values of three characters, namely total number of seeds per fruit, percentage of seed germination and seedling survival per fruit were recorded (Table I). Comparison among the four categories with respect to the three above characters showed that (A) control selfed and (D) *M<sub>1</sub>* selfed possessed higher and lower values, respectively, than both the respective reciprocal crosses. Statistical analysis (analysis of variances) showed that the reciprocal crosses (B and C) have nearly equal values whereas AB, AC, BD and CD values are significant at 1% level of probability for all characters at different exposures. The observations are similar to those of *L. cylindrica*<sup>3</sup> with reference to the significant and non-significant values of the respective crosses.

From the above results it is evident that both the gametes of *M<sub>1</sub>* plants of *L. acutangula* contribute equally and significantly towards total sterility, irrespective of one being numerically superior, more radiosensitive<sup>4</sup>, less viable<sup>5</sup> and subjected to

TABLE I  
Gametic damage as induced by gamma rays on *L. acutangula*

| Dose in kR | Category                          | % pollen fertility | Seeds/fruit No. S.E. | % Seed germination | Seedling/fruit No. S.E. |
|------------|-----------------------------------|--------------------|----------------------|--------------------|-------------------------|
| 20         | A—Control Selfed                  | 94                 | 106.2 ± 0.70         | 96                 | 99.2 ± 0.73             |
|            | B— <i>M<sub>1</sub></i> × Control | ..                 | 92.3 ± 0.16          | 87                 | 73.3 ± 1.50             |
|            | C—Control × <i>M<sub>1</sub></i>  | ..                 | 93.5 ± 1.13          | 86                 | 73.5 ± 1.53             |
|            | D— <i>M<sub>1</sub></i> Selfed    | 81                 | 82.4 ± 1.60          | 76                 | 54.4 ± 2.06*            |
| 30         | A—Control Selfed                  | 94                 | 106.2 ± 0.70         | 96                 | 99.3 ± 0.73             |
|            | B— <i>M<sub>1</sub></i> × Control | ..                 | 84.3 ± 1.40          | 78                 | 56.4 ± 1.83             |
|            | C—Control × <i>M<sub>1</sub></i>  | ..                 | 85.5 ± 1.36          | 77                 | 55.2 ± 1.90             |
|            | D— <i>M<sub>1</sub></i> Selfed    | 76                 | 71.4 ± 1.70          | 65                 | 31.6 ± 2.36*            |
| 40         | A—Control Selfed                  | 94                 | 106.2 ± 0.70         | 96                 | 99.2 ± 0.73             |
|            | B— <i>M<sub>1</sub></i> × Control | ..                 | 73.3 ± 1.73          | 64                 | 39.4 ± 2.23             |
|            | C—Control × <i>M<sub>1</sub></i>  | ..                 | 72.4 ± 1.70          | 65                 | 38.5 ± 2.30             |
|            | D— <i>M<sub>1</sub></i> Selfed    | 69                 | 52.3 ± 1.93          | 57                 | 18.3 ± 2.80*            |

\* Insignificant for all characters at each dose. Rest are significant at 1% level of probability.

competitive screening with reference to pollen lethality than the other.

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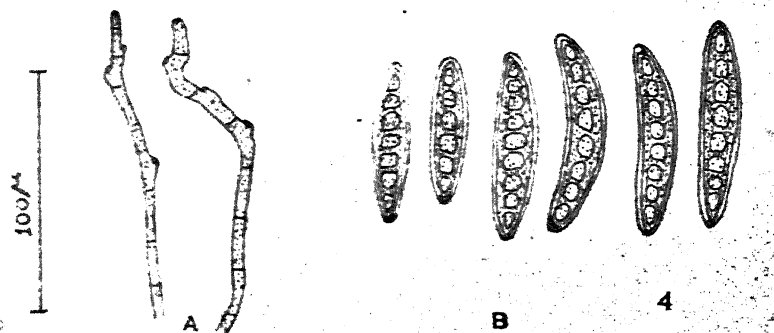
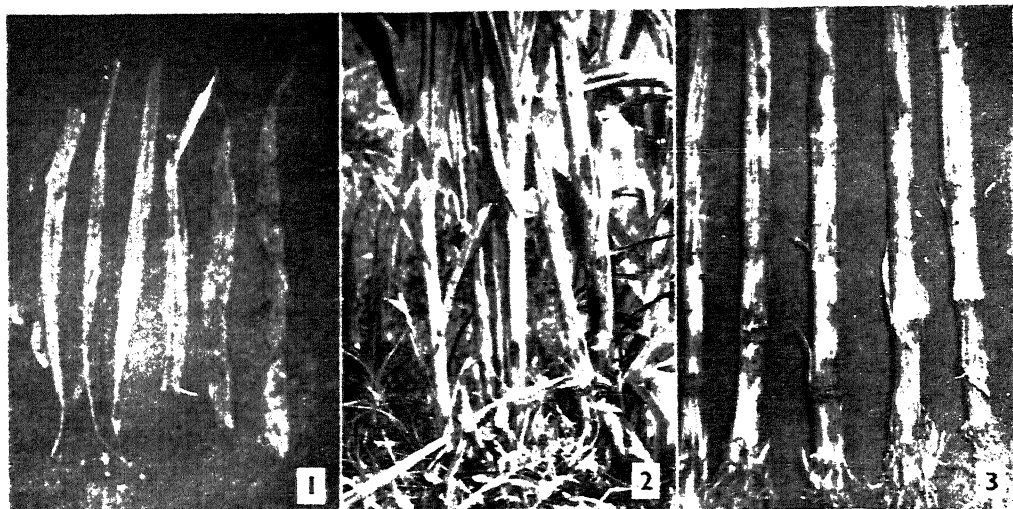
### A NEW MENACE TO "KAMADHENU", A HYBRID GRASS IN KARNATAKA

KAMADHENU, a new hybrid grass (*Pennisetum purpureum* Schum.  $\times$  *Pennisetum typhoides* Stapf.) is a recent release of University of Agricultural Sciences, Bangalore, and claimed to be a very

promising fodder source. It was released during 1972 and at a time when it is just getting popular with cultivators, it seems to have received a serious setback during 1974 due to widespread incidence of a fungal disease. There are reports from around Bangalore and Dharwar of total failure of the crop after first cutting, due to this disease. In view of the importance of this valuable hybrid in Agriculture and seriousness of this new malady, preliminary investigations were conducted to study the nature and cause of the disease.

Examination of the plants in different stages of the development of the disease has revealed that all the above-ground plant parts are involved.

On newly infected leaves, small reddish brown spots, surrounded by a yellow halo, appear. As these spots enlarge longitudinally and to a lesser extent in breadth, the centre of the spots becomes straw coloured with reddish brown margin. Majority of the spots have sharp ends resembling



FIGS. 1-3, 4 A-B

typical "eye-spots" (Fig. 1). Individual lesions on the leaves measure 0.5-1 cm in length and 3-5 mm in breadth. When such spots coalesce, they may measure upto 10-15 cm in length. Such coalesced spots do not have any well-defined shape or margin. In severe cases leaves show blighted appearance. Similar symptoms are found on leaf-sheaths, culms and at the collar region (Fig. 2). After first cutting, when the new tillers are emerging, they are badly damaged at the collar region resulting in death of the plants. Rotting at the collar region is not uncommon (Fig. 3).

Tissue isolations for the pathogen were made from the necrotised areas from leaves, culms and collar regions separately and all these isolations invariably yielded pure cultures of a fungus akin to *Helminthosporium* sp. Incidentally, it was noticed that cultures from such tissue isolations, when kept continuously in darkness in the incubator, did not sporulate. However when the same were exposed to alternate light and darkness, there was heavy sporulation.

A week old sporulating culture was successfully used to produce artificial infection on healthy plants of Kamadhenu grass and its parents, i.e., *P. purpureum* and *P. typhoides*. Typical symptoms were noticed on the leaves and leaf sheaths after 3 days of inoculation.

The fungus makes a fairly white growth on potato dextrose agar in the beginning, with age it turns dirty white and finally ashy brown. Abundant sporulation is observed in a week's time on P.D.A.

Conidiophores are straight or flexuous, dark-brown or olivaceous brown measuring  $119-200 \mu \times 2-8 \mu$  (Fig. 4A). Conidia are straight or slightly curved, narrowly ellipsoidal, pale brown to golden brown, measuring  $40-83 \mu \times 9-16 \mu$  (Average  $67 \mu \times 13 \mu$ ) with 5-9 pseudosepta (Fig. 4B).

20 days old culture on P.D.A. also produces abundant chlamydospores, which are usually spherical, intercalary or terminal and in chains.

Comparing the descriptions of the various species of *Helminthosporium* and *Drechslera* recorded on both the parents involved in the development of this hybrid grass, the fungus under consideration has been identified as *Drechslera sacchari* (Butler) Subram. and Jain. Diseased specimens and the culture of the fungus have been deposited in MYSP herbarium and culture collection of University of Agricultural Sciences, Hebbal, with accession No. MYSP 1902 and Culture No. 91 respectively.

Grateful thanks are due to Dr. H. C. Govindu, Senior Professor and Head, Department of Plant

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### EFFECT OF GAMMA IRRADIATION ON DIPLOID AND TETRAPLOID SEEDS OF *CAJANUS CAJAN* (L.) MILLSP.

SENSITIVITY of diploid and tetraploids to radiations and chemical mutagen treatments as measured by different criteria has been studied by several workers in different crop species<sup>1-3</sup>. In general poor germination and reduced seed fertility are associated with tetraploids and this could be improved by using radiation treatments to a considerable extent<sup>1-3</sup>. The present short communication describes radio-sensitivity of diploid and induced tetraploid seeds of *Cajanus cajan* (L.) Millsp. subjected to different doses of gamma irradiation.

Seeds of Colchicine induced tetraploids of variety T<sub>21</sub> in C<sub>4</sub> generation together with diploid parent were exposed to 0, 15, 20, 30, 40 and 60 Krad of gamma rays in a <sup>60</sup>Co source. After irradiation 100 seeds in each treatment were grown in enamel trays in two replications. Observations on germination (radical initiation) survival (per cent of seeds germinated), seedling height and number of leaves appeared on 21st day after sowing were recorded.

In diploids, germination, survival, seedling height and number of leaves were significantly reduced as the irradiation doses increased (Table I). While in tetraploids initial dose of 15 Krad significantly increased the germination as well as seedling height and the number of leaves. It is likely that low doses of irradiation break dormancy in tetraploids, by stimulating the favourable hormonal activity and cell membrane permeability.

As the irradiation doses increased above 15 Krad in tetraploid, there was a reduction in germination, survival seedling height and number of leaves. The C.D. value for ploidy and 2n x 4n interaction are also summarised in Table I.

TABLE I  
Germination, seedling survival height and number of leaves in diploid and tetraploid *Cajanus cajan* following different doses of gamma rays

| Gamma rays<br>in Krad | Germination<br>(%) |      | Survival<br>(%) |       | Height<br>(cm) |     | Number of<br>leaves |     |
|-----------------------|--------------------|------|-----------------|-------|----------------|-----|---------------------|-----|
|                       | 2n                 | 4n   | 2n              | 4n    | 2n             | 4n  | 2n                  | 4n  |
| 0                     | 97.5               | 45.5 | 100.0           | 100.0 | 14.7           | 7.0 | 3.7                 | 6.9 |
| 15                    | 91.0               | 91.5 | 93.4            | 98.2  | 8.3            | 9.6 | 3.7                 | 9.6 |
| 20                    | 88.5               | 88.5 | 89.3            | 96.0  | 7.7            | 9.5 | 3.6                 | 9.3 |
| 30                    | 59.0               | 60.0 | 57.6            | 74.1  | 7.1            | 6.6 | 3.0                 | 6.6 |
| 40                    | 40.5               | 40.0 | 39.5            | 42.5  | 3.5            | 5.7 | 3.0                 | 5.7 |
| 60                    | 27.0               | 06.0 | 5.5             | 17.1  | 3.0            | 3.0 | 2.2                 | 3.0 |
| C.D. 1% for ploidy    | 3.02               |      | 1.61            |       | 0.35           |     | 0.22                |     |

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### *Pennisetum polystachyum* (L.) SCHULT. A NEW CHROMOSOMAL RACE

THE genus *Pennisetum* is placed in the tribe Paniceae of Panicoideae—Poaceae. *Pennisetum polystachyum* (L.) Schult. is a polymorphic taxon comprising four chromosomal races— $2n = 36^{4*}$ ,  $2n = 48^2$ ,  $2n = 52^2$  and  $2n = 54^3$ . Hrishik has reported *P. polystachyum* ( $2n = 54$ ) to be a perennial grass while Bor<sup>1</sup> has described it as an annual.

The material for the present study was collected from Coimbatore, and grown in the departmental garden, University of Mysore, Mysore. Young florets of appropriate stages were fixed in 3:1 absolute alcohol-acetic acid solution and stored in 70% alcohol for meiotic studies. Pollen mother cell smears were prepared in acetocarmine. The mitotic chromosomes were studied following Tjio and Levan's<sup>2</sup> technique.

Morphological variations noticed in the present study of three chromosomal races could be positively correlated with the ploidy of the species. The

tetraploid ( $2n = 36$ ) is shorter with dark purplish inflorescence. The hexaploid ( $2n = 54$ ) is tallest with yellowish-brown inflorescence while the new chromosomal race ( $2n = 63$ ) is tall, the inflorescence being reddish-brown in colour.

The fifth chromosomal race of the present study is a new record with unbalanced chromosomes  $2n = 63$ . This has been confirmed by counting the somatic chromosomes from the root tips (Fig. 1).



FIGS. 1-2. Fig. 1. Somatic chromosomes of root tips.  $2n = 63$ .  $\times 665$ . Fig. 2. Meiosis—Diakinesis stage.  $\times 665$ .

This chromosomal race exhibits many abnormalities during meiosis. Multivalents and univalents occur in addition to bivalents during diakinesis (Fig. 2) and Metaphase I. In metaphase I bivalents and multivalents appear on the equatorial plate of the spindle and univalents being at periphery move precociously to the poles. Irregular spindles have been observed in the first and second meiotic divisions. The presence of laggards and their non-inclusion in the telophase nuclei result in micronuclei formation in dyads and tetrads. The irregular comportment of meiotic chromosomes during first and second divisions affect the pollen viability. Stainability tests further revealed that 56% of the pollen to be viable.

Cytoembryological studies of the three chromosomal races  $2n=36$ ,  $2n=54$  and  $2n=63$  indicate that they are facultative apomicts, the mechanism of reproduction being apospory. The details of cytoembryological studies and their reproductive behaviour will be published elsewhere. The observations made here agree with Bor<sup>1</sup> in confirming the species as annual.

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## PLANKTONIC FORAMINIFERA FROM THE BARIPADA BEDS, ORISSA

A DETAILED micropaleontological investigation of the Baripada beds, exposed in the cuttings of the river Burhabalanga, south of the town Baripada ( $21^{\circ} 56' N : 86^{\circ} 44' E$ ) in Mayurbhanj District, Orissa, reveals the presence of a fairly well-preserved foraminiferal assemblage which includes three planktonic species. A comprehensive study pertaining to the systematics, paleoecology and age of foraminifera is in progress and will be published in due course. However, since the planktonic foraminifera are valuable indicators of age, it was considered desirable to report them in the form of present note so that they may illuminate the polemical age of the Baripada beds.

Very little is known about the foraminifera from the Baripada beds. Amongst planktonic forms, only *Globigerina* sp. and *Globorotalia* sp. have been reported by Sarma<sup>1</sup>, Tewari and Awasthi<sup>2</sup>, and Chatterjee<sup>3</sup>. In our material, the planktonic foraminifera occur in very low frequencies and are represented by the following three species: (1) *Globorotalia* sp. indet, (2). *Globigerina* cf. *G. quadriloba* d'Orbigny, (3) *Orbulina suturalis* Brönnimann.

*Orbulina suturalis* makes its first appearance in a limestone band, sandwiched between shale beds of Baripada beds. It is a well-known age marker and was originally described by Brönnimann<sup>4</sup> from the *Globorotalia menardii* zone in the Miocene of Trinidad. A comparative study of the specimens of *O. suturalis* from Baripada was made with the material of this species from Trinidad, kindly sent by Dr. W. Weaver, U.S.A. Our specimens were found identical to the forms from Trinidad in showing the presence of Globigerine-structure as well as faintly developed rounded pores along sutures, separating the Globigerine part from the final chamber, and also sparsely scattered around it, in addition to tiny pores all over the test. The genus *Globigerina* d'Orbigny is represented by a solitary and poorly preserved specimen comparable to *Globigerina quadriloba* d'Orbigny, as illustrated by Bandy<sup>5</sup>. This species was originally described from marly plastic clays (Vindobonian) from Austria. Another genus of planktonic foraminifera is represented by a few specimens of *Globorotalia* Cushman but their poor state of preservation does not permit valid specific identification.

A study of literature indicates that the first appearance of *Orbulina suturalis* is significant in age determination. A majority of foraminiferalogists consider that it first appears during Helvetian stage of Europe and marks the base of Helvetian in Australia and Lillburnian stage in New Zealand, i.e., the first appearance of *O. suturalis* indicates the base of Middle Miocene (personal communications, Dr. D. G. Jenkins, New Zealand, and Dr. N. H. Ludbrook, Australia).

The presence of *Orbulina suturalis* and *Globigerina quadriloba* in the Baripada beds suggests a Middle Miocene age for these beds but in the absence of other marker species, no precise placement within the Middle Miocene could be made.

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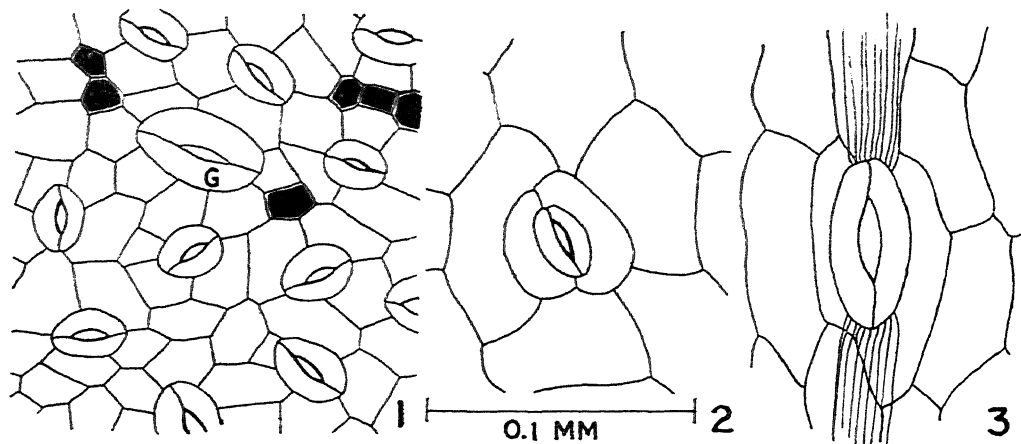
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# OCURRENCE OF GIANT STOMATA IN CELASTRACEAE AND CONVULVULACEAE

GIANT stomata occur on certain leaves along with the normal stomata<sup>1</sup>. There are a few reports on the occurrence of giant stomata<sup>1-6</sup>. While studying the epidermal features in Celastraceae and Convolvulaceae, we observed abnormally large stomata on the leaves of *Celastrus stylosus* Wall., *Euonymus hamiltonianus* Wall. and *Hippocratea arborea* Roxb. (Celastraceae), and *Ipomoea aquatica* Forsk. (Convolvulaceae).

Stace<sup>1</sup> reported their rare occurrence on the veins and venules. The giant stomata on the midrib and larger veins are in the process of degeneration. However, the ones on the venules show distinct stomatal pores and guard cells comparable to normal stomata. In all the species studied, the giant stomata are almost twice as large as the normal stomata (Table 1, Figs. 1-3). In addition to their size, in *I. aquatica*, the giant stomata can be distinguished from normal, by the presence of two lateral groups of striae (Fig. 3).



FIGS. 1-3. Fig. 1. A portion of lower epidermis of *Hippocratea arborea* in surface view showing a giant stomata (G) along with several stomata of normal size. Figs. 2, 3. A normal stomata from intercostal area and a giant stomata from costal area respectively in surface view from lower epidermis of *Ipomoea aquatica*.

TABLE I

| Species                    | Size of stomata (L × B) in $\mu$ |                | Stomatal frequency per sq. mm | Stomatal index |
|----------------------------|----------------------------------|----------------|-------------------------------|----------------|
|                            | Normal                           | Giant          |                               |                |
| 1. <i>C. stylosus</i>      | 23-25<br>20-46                   | 39-99<br>25-11 | 342                           | 14.16          |
| 2. <i>E. hamiltonianus</i> | 25-11<br>23-25                   | 39-06<br>26-37 | 369                           | 18.75          |
| 3. <i>H. arborea</i>       | 19-53<br>15-81                   | 45-57<br>29-76 | 368                           | 20.06          |
| 4. <i>I. aquatica</i>      | 18-52<br>7-63                    | 41-16<br>18-52 | U: 125<br>L: 250              | 9.23<br>16.62  |

U—upper surface of leaf, L—lower surface of leaf.

The leaves are hypostomatic in Celastraceae, and amphistomatic in Convolvulaceae. In the former, giant stomata occur on the lower surface of leaf and in the latter on both the surfaces. They occur on the midrib, veins, venules, and areoles, though

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Meerut, August 5, 1974.

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## FLUORESCENT ANTIBODY TEST FOR DETECTION OF CITRUS GREENING MYCOPLASMA

In medical sciences the fluorescent antibody technique has been widely used in visualization and identification of bacteria and viruses<sup>2</sup>, and to some extent of mycoplasma<sup>3</sup>. Also, some enzymes and

hormones have been traced in animal tissues by this method<sup>2</sup>. However, very little work has been done in the field of plant pathology<sup>4-6</sup>; none dealing with plant mycoplasmas.

In the present studies, culture of mycoplasma obtained earlier from greening affected citrus plants on PPLO broth<sup>7</sup>, served as antigen. It was concentrated by one cycle of differential centrifugation which involved centrifugation at 10,000 rpm for 20 minutes in a Spinco Ultracentrifuge Model L using phosphate buffer (pH 7.0) to separate the suspended impurities, followed by high speed centrifugation at 25,000 rpm for 120 minutes and dissolving the pellet in a small quantity of M/30 phosphate buffer before final centrifugation at 5,000 rpm for 15 minutes. The concentrated antigen was injected in rabbits intramuscularly thrice at weekly intervals with Freund's adjuvant followed by one intravenous injection without the adjuvant. The blood, collected by bleeding the rabbits after 10 days following the last injection, was allowed to clot at room temperature for four hours and stored overnight in a refrigerator for clot to shrink. The serum was decanted and clarified by low speed centrifugation (7,000 rpm for 15 minutes). The antiserum reacted specifically with the mycoplasma culture and had a titre of 1 : 512.  $\gamma$ -globulins were extracted from antiserum by precipitating with equal volume of 3.2 N ammonium sulphate, dissolving the precipitate in 2 ml *tris*-HCl buffer (pH 7.2) and reprecipitating repeatedly until the precipitate was absolutely white. The  $\gamma$ -globulin solution was dialysed against the same buffer until all the sulphate ions were removed. The pH of the solution was raised to 8.5 with 0.5 M carbonate-bicarbonate buffer (pH 9.0) before stirring it with fluorescein isothiocyanate (FITC), added to the solution at the rate of 50 mg per g of protein, for 4-6 hours in cold (4° C). The preparation was then eluted with the *tris*-HCl buffer on a Sephadex (G-25) column to remove the unconjugated dye (FITC).

For detection of mycoplasma, sections of infected as well as uninfected leaves were cut and flooded with *tris*-HCl buffer. They were then transferred to slides and each one was covered with 1-2 drops of FITC conjugated antiserum. The slides were left in a moist chamber for 8-12 hours at room temperature. After draining off excess conjugated antiserum, the sections were washed with *tris*-HCl buffer and mounted in *tris*-HCl buffered glycerine. When examined under the fluorescent microscope, sections of healthy leaves showed dull blue autofluorescence all over except in the thick walled cells which fluoresced in light green range. In infected leaves, in addition to autofluorescence noted in the healthy leaves, brilliant apple green fluorescence in

the form of dots was observed in phloem cells (Fig. 1). This indicated the presence of mycoplasma.

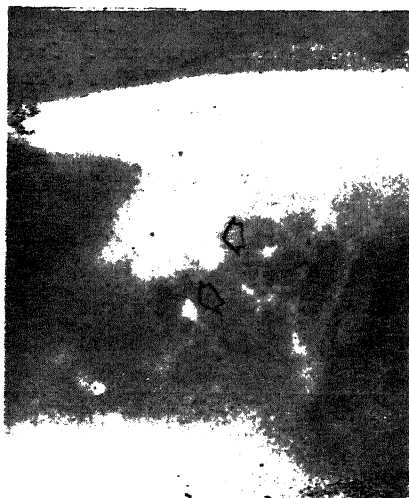


FIG. 1. Fluorescence photomicrograph of greening affected citrus phloem cell showing mycoplasma-like pathogen (arrows) stained with fluorescein-conjugated antiserum.  $\times$  1,200, approx.

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|                                 |                     |
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| Institute,                      | T. K. NARIANI.      |
| New Delhi 110012, July 3, 1974. |                     |

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# TOXIN PRODUCTION BY CORYNEBACTERIUM CASSICOLO AND CURT WIL

A fungus, *Corynebacterium cassicola* (Curt) Wilt, has been found to cause wilt and leaf death in brinjal by producing a necrotic spot syndrome. *C. cassicola* (Curt) Wilt and Curt Wilt is a non-specific wilt pathogen which causes leaf death of many plants including brinjal. The leaf spot phase is a minute water soaked area which expands in size and later develops necrosis, often accompanied by a typical chlorotic halo. Because of the sequential development of symptoms, toxin production by the fungus was suspected. Results of a preliminary investigation are reported herein.

and seven in the same sequence. All the organic fractions were evaporated to dryness. The individual fractions from the CF and mycelium were water insoluble and, therefore, suspended in water and used for biological activity employing the leaf spot bioassay. The results of the bioassay are given in Table I.

The earliest symptom was water soaking at the point of application on the lower surface of leaf. Eventually there developed on the upper surface of the leaves a necrotic lesion with a chlorotic halo, simulating the natural lesion. This stage is referred to as the complete symptom. Out of all the fractions benzene fraction and ethylacetate fractions gave most biological activity. The appearance of necrotic symptoms with fractions from the fungus

TABLE I

Effect of various fractions of the culture filtrate and mycelium (CF) of *C. cassicola* on brinjal leaves

| Solvent fraction | Time required for the symptom development |          |  |          |
|------------------|---|----------|--|----------|
|                  | First appearance                          |          | Complete symptom development                               |          |
|                  | CF  | Mycelium | CF   | Mycelium |
| Petroleum ether  | 65-72 hrs.                                | ..       | Chlorosis is not seen<br>Necrosis noticed after<br>72 hrs. | ..       |
| Benzene          | 2-3 min.                                  | 2-5 mts. | 48 hrs.  | 48 hrs.  |
| Chloroform       | 68 hrs.                                   | 48 hrs.  | 96 hrs.  | 72 hrs.  |
| Ethyl acetate    | 36 hrs.                                   | 36 hrs.  | 72 hrs.  | 72 hrs.  |

The fungus was grown in still liquid cultures, the medium consisting of 3.3% sucrose, 0.33%  $\text{KH}_2\text{PO}_4$ , 0.17%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.1% casein hydrolysate adjusted to pH 5.5. Cell free culture filtrate (CF) of 10-day old cultures were obtained by filtration through Whatman No. 1 filter paper. Immersing petioles of detached leaves and cut shoots of brinjal (cut leaf/shoot bioassay) in CF induces flaccidity of leaves, and wilt in cut shoots in 4 hours. Cut shoots of tobacco, *Solanum nigrum* L. and castor also showed similar symptoms when kept in CF. Necrotic spots developed in 4 days at the points of application of CF on the lower surface of detached brinjal leaves incubated in humid petri plates (leaf spot bioassay).

The CF was concentrated *in vacuo* and precipitated with ethanol. The supernatant was serially extracted with petroleum ether, benzene, chloroform and ethyl acetate. The fractions were later evaporated to dryness. Freshly harvested mycelial mats were washed thoroughly with glass distilled water and blotted with Whatman No. 1 filter papers. These mats were later extracted with

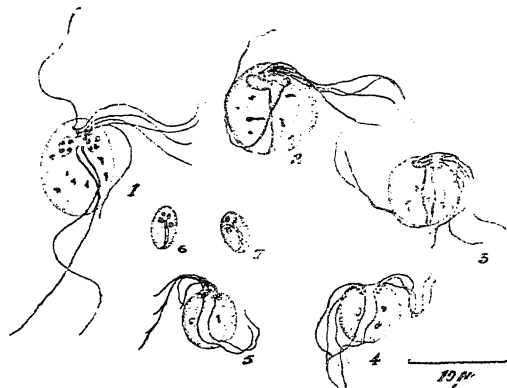
and CF indicates that the toxic metabolites are endogenous and are secreted into the medium during its active growth phase. Appearance of wilt symptoms in cut shoots of brinjal and certain other hosts and production of necrotic spots with toxic fractions on leaves of brinjal and certain unrelated hosts suggest that the substance involved is of non-specific phytotoxic nature, which is probably related to the ability of the fungus to infect a large variety of plants. Further work on toxin purification is in progress.

Department of Botany, Y. R. SARMA,  
S.V. University, Tirupati (A.P.), M. V. NAYUDU.  
August 16, 1974.

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THE MORPHOLOGY OF *HEXAMITUS*  
*KAKATHIAE* N. SP. FROM GARDEN LIZARD  
*CALOTES VERSICOLOR*

THE investigation of the intestinal Protozoans of the lizards of Warangal, A.P., revealed an interesting flagellate belonging to the genus *Hexamitus* Dujardin<sup>1</sup>. This genus belongs to the family Hexamitidae Kent and to the order D'plomonadida Wenyon<sup>2</sup>. The infestation was very heavy and found in 85% of the lizards. Extensive work carried out by K. Janaki Devi<sup>3</sup> on Andhra Pradesh reptilian fauna, described a new species from rock lizard, *Calotes nemoricola*. The new species has a typical spherical shape both in living as well as in stained preparations. It measures  $8.0\mu$  to  $8.5\mu$  in diameter. The blepharoplast complex is somewhat interesting (Figs. 2-5). In few forms all the blepharoplasts are fused as a rod-like structure and are placed at the anterior region of the two nuclei (Fig. 1).



FIGS. 1-7. *Hexamitus kakathiae* n. sp. Figs. 1, 4 and 5. Round forms, with four plaques in the nucleus. These figures are drawn from the material fixed in Scheudinn's and stained with iron haematoxylin. Figs. 2 and 3 are showing acronemes to the flagella and are fixed in methylalcohol, stained with Giemsa's. Figs. 6-7 are cysts.

From these complex blepharoplasts three anterior flagella arise, which are subequal in length. In most cases the anterior flagella are ending in acronemes (Figs. 1-4). Axial structures are rod-like and are separated comparatively to a greater extent (Figs. 4-5). However, in a few forms they are

crossed (Figs. 1-2). The axostyles continue beyond the body as the posterior flagella which run close together, and end in acronemes (Figs. 1-3). The two nuclei are round masses, associating to form a single lobe. In most cases these nuclei possess large chromatin granules (Fig. 1). Cysts are very small measuring  $3.5\mu$  in diameter and in almost all cases they are oval in shape (Figs. 6-7).

The genus *Hexamitus* founded by Dujardin<sup>1</sup> includes 3 species. The species *H. xenopi* Fantham and Por<sup>4</sup> and *H. natrix* Matubayashi<sup>5</sup> are reported from opsidan hosts. The third one, *H. kirby*, was reported by Janaki Devi<sup>3</sup> (1957) from rock lizard *Calotes nemoricola*.

The species *H. kirby* Janaki Devi differs in many of the characters from the new species described here from a new host *Calotes versicolor*. *H. kirby* differs in size ( $6.5\mu$ - $13.0\mu$ )  $8.7\mu \times (3.8\mu$ - $10.0\mu)$   $6.6\mu$ . The nuclei are round in shape with central endosome; the six anterior flagella are equal in length; two clear blepharoplasts are at the anterior end of the nuclei; the axial structures possess a chromatic ring at the posterior end.

In view of these differences the parasite under discussion is considered to be a new species and is designated *Hexamitus kakathiae* n. sp. after the place Kakathia, Warangal, A.P.

I wish to express my sincere thanks to Prof. S. S. Qadri, Head, Department of Zoology, Osmania University, Hyderabad, for guidance and encouragement. I am also thankful to Prof. P. Ramchender Rao for Laboratory facilities and encouragement.

Department of Zoology, T. BHASKAR RAO.  
Post-Graduate Centre,  
Warangal-1, A.P., India.  
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# DOWNY MILDNESS IN C. RINOVORCA IN HOT CLIMATE AND CURE

Downy mildew of *C. rinovorca* is a serious disease of this crop in the hot climate of the coastal belt of the Andaman Islands. It is caused by the fungus *Peridermium cinereum* (W.) Sacc. The disease is characterized by the appearance of white, fuzzy growth on the lower surface of the leaves, which later turns brown and necrotic.

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TABLE I

Time taken for the appearance of the symptoms of downy mildew on brinjal leaves

Time taken for the appearance of the symptoms of downy mildew

| Solvent fraction | First appearance |          | Complete symptom development                             |          |
|------------------|------------------|----------|--|----------|
|                  | CF               | Mycelium | CF   | Mycelium |
| Petroleum ether  | 25-32 hrs        |          | Chlorosis is not seen<br>Necrosis 72 hrs after<br>72 hrs |          |
| Benzene          | 2-5 min          | 2-5 min  | 48 hrs   | 48 hrs   |
| Chloroform       | 48 hrs           | 48 hrs   | 96 hrs   | 72 hrs   |
| Ethyl acetate    | 36 hrs           | 36 hrs   | 72 hrs   | 72 hrs   |

The fungus was grown in well liquid cultures the medium consisting of 3.3% sucrose, 0.33%  $\text{KH}_2\text{PO}_4$ , 0.17%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.17% yeast hydrolysate adjusted to pH 5.5. Cell free culture filtrate (CF) of 10-day old cultures were obtained by filtration through Whatman No. 1 filter paper. Immersing petioles of detached leaves and cut shoots of brinjal (cut leaf shoot bioassays) in CF induces flaccidity of leaves, and wilt in cut shoots in 4 hours. Cut shoots of tobacco, *Solanum nigrum* L. and castor also showed similar symptoms when kept in CF. Necrotic spots developed in 4 days at the points of application of CF on the lower surface of detached brinjal leaves incubated in humid petri plates (leaf spot bioassays).

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5. Honigberg, B. M. *et al.*, *J. Protozoology*, 1964, 11, 7.

## SHORT SCIENTIFIC NOTES

### On the Occurrence of *Blumea heiracifolia* (D. Don) DC. var. *flexuosa* (Cl.) Randeria (Asteraceae) in India

During a recent exploration for the collection of medicinal plants in Chikmagalur district the authors collected near Sirilu village, 40 km from Sringeri (Karnataka State) a taxon identified as *Blumea flexuosa* C.B.Cl.

The revision work of *Randeria* in *Blumea* 10 (1) : 1960, 176-317, on the genus *Blumea* DC. was consulted for determining the nomenclature of this taxon. It was observed that *Randeria*, l.c., p. 249, has reduced this species *flexuosa* C.B.Cl. to a variety under *Blumea heiracifolia* (D. Don) DC. var. *flexuosa* (Cl.) *Randeria*, effecting the new combination also. Further, the occurrence of this plant has been stated to be 'endemic' to Ceylon. This observation of endemism has been based on the herbarium specimens examined by her.

The authors examined the type material at MH and one sheet of *Thwaites* C.P. 19 was observed to be present among this type material and the specimens collected by the authors exactly matched with this specimen. Further, there was one more sheet under *B. flexuosa* collected by R.H. Beddome s.n. (accession No. 27641) with the locality mentioned as South Canara which also matches with the specimen present *Thwaites* C.P. 19.

Gamble has annotated in pencil on this sheet: "I am not sure this may not be *B. crinita*". But *B. crinita* can be easily distinguished from *B. heiracifolia* var. *flexuosa* in not being woolly or silky, having denticulated leaf margins and rounded leaf bases (*Randeria*, l.c., p. 251).

The collection of our specimens from Sirilu village and the presence of a sheet in MH from South Canara, both of which match with the type material *Thwaites* C.P. 19 present at MH, corroborates the occurrence of this taxon as mentioned by Gamble in the *Flora of the Presidency of Madras*, p. 481 (repr. ed. 1967) under *B. flexuosa*, as occurring in "Western Ghats, hills of Mysore at 3,500 ft., Sispara in Nilgiris about 6,000 ft., hills of Travancore at 6,000 ft".

While *Randeria* has made every attempt to examine all available sheets in the various herbaria, it is quite possible that she has missed the sheets from South India (since no reference has been made in the revision work) on which Gamble, l.c., p. 481 has based the occurrence of this taxon. Since all the sheets referred to by *Randeria* are from Ceylon,

it has led her to the conclusion that the taxon is endemic to Ceylon.

Our recent collections, supported by a sheet present in MH from South Canara and also the distribution of the taxon as mentioned by Gamble (l.c.) are sufficient to prove that *B. heiracifolia* (Don) DC. var. *flexuosa* (Cl.) *Randeria* is not endemic to Ceylon but also occurs in South India.

*Herbarium specimens examined*: MH: *Thwaites* C.P. 19: one sheet with accession No. 27662 (from Ceylon); R. H. Beddome s.n. (accession No. 27641 from South Canara, 1867). *Yoganarasimhan* 1109, 22-2-1972, flowering, near Sirilu village, ca 1,000 m. fairly common in grasslands of hill tops, deposited at the Herbarium of the Regional Research Centre, Bangalore-11.

The authors are thankful to Mr. Vivekananthan, Botanist, for useful discussion and to the Regional Botanist, Botanical Survey of India, Coimbatore, for permitting to consult the Madras herbarium. Thanks are also due to the Director, Central Council for Research in Indian Medicine and Homoeopathy, New-Delhi and Officer-in-Charge, Regional Research Centre, Bangalore, for facilities.

Regional Research Centre S. N. YOGANARASIMHAN.  
(C.C.R.I.M.H.). K. SUBRAMANYAM.  
Bangalore-11. October 23, 1974.

### Discovery of Uraniferous Precambrian Conglomerates at Chikmagalur, Karnataka, India

*Introduction*.—The purpose of this note is to report the discovery of uranium-bearing conglomerates of Precambrian age at Chikmagalur (13° 19' 20" N. 75° 46' 30" E.; Survey of India top-sheet No. 480/15) in Karnataka State, and to briefly describe their geological setting, radioactivity, and mineralogy.

*Geological setting*.—The uraniferous conglomerates occur over a granitic-migmatitic basement, and grade upwards to pebbly quartzite, grit, arkose, and quartzite. This sequence is followed by a second zone of conglomerates, and by massive mafic metavolcanic rocks, in some of which pillow structures can be recognised.

*Uranium content*.—Surface samples of the conglomerates have assayed upto 0.13%  $U_3O_8$ , and core samples from boreholes drilled to a depth of 106 metres analyse upto 0.21% uranium. 90% of the uranium is in a leachable state.

**Distribution.**—The uraniferous conglomerates occur as five distinct ribbon-shaped bands or beds, each 5 km long.

**Mineralogy.**—The radioactive minerals in the conglomerates are pitchblende, monazite, and zircon. The other interesting constituents are chalcopyrite, covellite, rammelsbergite, pyrite, siderite, ilmenite, rutile, leucoxene, and magnetite. The common minerals include white and grey quartz pebbles (1 to 3 cm in diameter) embedded in a quartz-rich matrix, and some ferromagnesian assemblages.

**Conclusion.**—Preliminary studies suggest that the uraniferous conglomerates of Chikmagalur are comparable to the well-known uranium-rich Precambrian quartz-pebble conglomerates of Blind River in Canada, and the Witwatersrand in South Africa. The results of detailed investigations currently in progress will be published elsewhere.

I am grateful to Dr. G. R. Udas, Director, Atomic Minerals Division, for suggesting the study.

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# Identification of Chlorinated Hydrocarbon Pesticides on T.L.C. Plates Using Modified Wood's Reagent

Wood<sup>1</sup> described the use of a mixture of bromophenol blue and silver nitrate for the detection of chloride ion on paper chromatograms. Abbott, Egan and Thomson<sup>2</sup> used this reagent for the detection of chlorinated hydrocarbon pesticides on T.L.C. plates. However the limits of detectability were not reported. Their method of detection involved several steps and there was difficulty in handling the plates at 105° C.

A modified Wood's reagent using ammonia vapour exposure, developed in this laboratory, eliminates these difficulties and facilitates detection of chlorinated pesticides at levels less than 1 µg. The method is as follows :

T.L.C. plates are prepared using prewashed silica gel G with a layer thickness of 250 µ. Chromatograms of Aldrin, Dieldrin, Endrin, Thiodan and D.D.T. with different concentrations are developed using cyclo-hexane as solvent. After development the plates are dried at room temperature and sprayed with the modified Wood's reagent (90 ml of 0.05% bromophenol blue dissolved in acetone plus 10 ml of 2% aqueous silver nitrate solution). They are then exposed to ammonia vapour for 15 minutes in a sealed tank. Blue spots on purple background

are observed. The limits of detectability of various pesticides are presented in Table I.

TABLE I

| Pesticide          | Lowest limit of detectability (µg) | Value of $\lambda_{max}$ (nm)            |
|--------------------|------------------------------------|--|
| Aldrin             | 0.5                                | 0.22                                     |
| Dieldrin           | 0.5                                | 0.29                                     |
| Endrin             | 0.5                                | 0.31                                     |
| Thiodan            | 3.0                                | 0.38                                     |
| OP, DPT and PP DDT | 0.5 µg/in technical DDT            | 0.75 for OP isomer<br>0.72 for PP isomer |

Central Tobacco C. V. S. S. V. G. GOPAKRISHNA  
Research Institute, A. S. SASTRY  
Rajahmundry 533101, N. C. GOPALACHARI  
Andhra Pradesh,  
August 9, 1974

1. Wood, T., *Nature*, 1955, 176, 175.
2. Abbott, D. C., Egan, H. and Thomson, J., *J. Chromatogr.*, 1964, 16, 481.

## *Archipsocus* sp. (Archipsocidae: Psocoptera), A New Pest of Citrus from India\*

Some Mandarin (*Citrus reticulata*) trees were found severely attacked by psocid, *Archipsocus* sp. during the surveys of citrus orchards in Coorg District during September–October 1972 and 1973. Nymphs and adults gregariously feed on the foliage and shoots by inhabiting within the silken webs formed by them. Occasionally the colonies extend upto the main branches. Affected twigs turn yellow and chlorotic. In severe cases the leaves shed off and twigs and branches die out. About 128 insect pests, so far, have been reported to attack *Citrus* spp. in India<sup>1</sup>. This is the first record of this insect pest on citrus and as such is an addition to the above list.

Thanks are due to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, for encouragement and to Dr. R. G. Fennah, Director, British Museum, London, for identification of the pest.

Indian Institute of Horticultural Research, Bangalore-6, V. G. PRASAD,  
K. C. SRIVASTAVA,  
K. KRISHNANAH.  
November 3, 1974.

\* Contribution No. 343 of Indian Institute of Horticultural Research, 255, Upper Palace Orchards, Bangalore-6.

1. Pruthi, H. S. and Mani, M. S., *ICAR, Sci. Monogr. No. 16*, 1945.

## SHORT SCIENTIFIC NOTES

### On the Occurrence of *Blumea heircifolia* (D. Don) DC. var. *flexuosa* (Cl.) Randeria (Asteraceae) in India

During a recent exploration for the collection of medicinal plants in Chikmagalur district the authors collected near Sirilu village, 40 km. from Sringeri (Karnataka State) a taxon identified as *Blumea flexuosa* C.B.Cl.

The revision work of Randeria in *Blumea* 10 (1) : 1960, 176-317, on the genus *Blumea* DC. was consulted for determining the nomenclature of this taxon. It was observed that Randeria, *l.c.*, p. 249, has reduced this species *flexuosa* C.B.Cl. to a variety under *Blumea heircifolia* (D. Don) DC. var. *flexuosa* (Cl.) Randeria, effecting the new combination also. Further, the occurrence of this plant has been stated to be 'endemic' to Ceylon. This observation of endemism has been based on the herbarium specimens examined by her.

The authors examined the type material at MH and one sheet of Thwaites C.P. 19 was observed to be present among this type material and the specimens collected by the authors exactly matched with this specimen. Further, there was one more sheet under *B. flexuosa* collected by R.H. Beddome s.n. (accession No. 27641) with the locality mentioned as South Canara which also matches with the specimen present Thwaites C.P. 19.

Gamble has annotated in pencil on this sheet: "I am not sure this may not be *B. crinita*". But *B. crinita* can be easily distinguished from *B. heircifolia* var. *flexuosa* in not being woolly or silky, having denticulated leaf margins and rounded leaf bases (Randeria, *l.c.*, p. 251).

The collection of our specimens from Sirilu village and the presence of a sheet in MH from South Canara, both of which match with the type material Thwaites C.P. 19 present at MH, corroborates the occurrence of this taxon as mentioned by Gamble in the *Flora of the Presidency of Madras*, p. 481 (repr. ed. 1967) under *B. flexuosa*, as occurring in "Western Ghats, hills of Mysore at 3,500 ft., Sispara in Nilgiris about 6,000 ft., hills of Travancore at 6,000 ft".

While Randeria has made every attempt to examine all available sheets in the various herbaria, it is quite possible that she has missed the sheets from South India (since no reference has been made in the revision work) on which Gamble, *l.c.*, p. 481 has based the occurrence of this taxon. Since all the sheets referred to by Randeria are from Ceylon,

it has led her to the conclusion that the taxon is endemic to Ceylon.

Our recent collections, supported by a sheet present in MH from South Canara and also the distribution of the taxon as mentioned by Gamble (*l.c.*) are sufficient to prove that *B. heircifolia* (Don) DC. var. *flexuosa* (Cl.) Randeria is not endemic to Ceylon but also occurs in South India.

*Herbarium specimens examined*: MH: Thwaites C.P. 19; one sheet with accession No. 27662 (from Ceylon); R. H. Beddome s.n. (accession No. 27641 from South Canara, 1867). Yoganarasimhan 1109, 22-2-1972, flowering, near Sirilu village, ca 1,000 m. fairly common in grasslands of hill tops, deposited at the Herbarium of the Regional Research Centre, Bangalore-11.

The authors are thankful to Mr. Vivekananthan, Botanist, for useful discussion and to the Regional Botanist, Botanical Survey of India, Coimbatore, for permitting to consult the Madras herbarium. Thanks are also due to the Director, Central Council for Research in Indian Medicine and Homoeopathy, New-Delhi and Officer-in-Charge, Regional Research Centre, Bangalore, for facilities.

Regional Research Centre S. N. YOGANARASIMHAN,  
(C.C.R.I.M.H.), K. SUBRAMANYAM,  
Bangalore-11, October 23, 1974.

### Discovery of Uraniferous Precambrian Conglomerates at Chikmagalur, Karnataka, India

*Introduction*.—The purpose of this note is to report the discovery of uranium-bearing conglomerates of Precambrian age at Chikmagalur (13° 19' 20" N. 75° 46' 30" E.; Survey of India top-sheet: No. 480/15) in Karnataka State, and to briefly describe their geological setting, radioactivity, and mineralogy.

*Geological setting*.—The uraniferous conglomerates occur over a granitic-migmatitic basement, and grade upwards to pebbly quartzite, grit, arkose, and quartzite. This sequence is followed by a second zone of conglomerates, and by massive mafic metavolcanic rocks, in some of which pillow structures can be recognised.

*Uranium content*.—Surface samples of the conglomerates have assayed upto 0.13%  $U_3O_8$ , and core samples from boreholes drilled to a depth of 106 metres analyse upto 0.21% uranium. 90% of the uranium is in a leachable state.

**Distribution.**—The uraniferous conglomerates occur as five distinct ribbon-shaped bands or beds, each 5 km long.

**Mineralogy.**—The radioactive minerals in the conglomerates are pitchblende, monazite, and zircon. The other interesting constituents are chalcocopyrite, covellite, rammelsbergite, pyrite, siderite, ilmenite, rutile, leucocene, and magnetite. The common minerals include white and grey quartz pebbles (1 to 3 cm in diameter) embedded in a quartz-rich matrix, and some ferromagnesian assemblages.

**Conclusion.**—Preliminary studies suggest that the uraniferous conglomerates of Chikmagalur are comparable to the well-known uranium-rich Precambrian quartz-pebble conglomerates of Blind River in Canada, and the Witwatersrand in South Africa. The results of detailed investigations currently in progress will be published elsewhere.

I am grateful to Dr. G. R. Udas, Director, Atomic Minerals Division, for suggesting the study.

Atomic Minerals Division, B. V. RAMA RAO,  
Department of Atomic Energy,  
Patan Bhavan, Race Course Road,  
Bangalore 560001, December 20, 1974.

#### Identification of Chlorinated Hydrocarbon Pesticides on T.L.C. Plates Using Modified Wood's Reagent

Wood<sup>1</sup> described the use of a mixture of bromophenol blue and silver nitrate for the detection of chloride ion on paper chromatograms. Abbott, Egan and Thomson<sup>2</sup> used this reagent for the detection of chlorinated hydrocarbon pesticides on T.L.C. plates. However the limits of detectability were not reported. Their method of detection involved several steps and there was difficulty in handling the plates at 105° C.

A modified Wood's reagent using ammonia vapour exposure, developed in this laboratory, eliminates these difficulties and facilitates detection of chlorinated pesticides at levels less than 1 µg. The method is as follows:

T.L.C. plates are prepared using prewashed silica gel G with a layer thickness of 250 µ. Chromatograms of Aldrin, Dieldrin, Endrin, Thiodan and D.D.T. with different concentrations are developed using cyclo-hexane as solvent. After development the plates are dried at room temperature and sprayed with the modified Wood's reagent (90 ml of 0.05% bromophenol blue dissolved in acetone plus 10 ml of 2% aqueous silver nitrate solution). They are then exposed to ammonia vapour for 15 minutes in a sealed tank. Blue spots on purple background

are observed. The limits of detectability for various pesticides are given in Table I.

TABLE I

| Pesticide      | Lowest Detectability (µg) | Limit of detection (1:4 to 1:1) |
|----------------|---------------------------|---------------------------------|
| Aldrin         | 0.5                       | 0.05                            |
| Dieldrin       | 0.5                       | 0.25                            |
| Endrin         | 0.5                       | 0.31                            |
| Thiodan        | 3.0                       | 0.38                            |
| OP, DPT and PP | 0.6 µg/in                 | 0.75 for OP isomer              |
| DDT            | technical DDT             | 0.75 for PP isomer              |

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Indian Institute of V. G. PRASAD,  
Horticultural Research, K. C. SRIVASTAVA,  
Bangalore-6, K. KRISHNAIAH,  
November 3, 1974.

\* Contribution No. 343 of Indian Institute of Horticultural Research, 255, Upper Palace Orchards, Bangalore-6.

1. Pruthi, H. S. and Mani, M. S., *ICAR, Sci. Monogr. No. 16*, 1945.



### Test of Baermann Technique for the Recovery of Infective Larvae of *Cooperia curticei* from Faecal Pellets

In ecological and epidemiological studies of strongyle nematodes the recovery of the infective larvae from cultures of faecal pellets may be necessary. Infective larvae are generally recovered from faecal pellets by Baermann technique; but the efficiency of this technique may depend on the bulk and physical conditions of the faecal culture. A test of this technique was done with known number of *Cooperia curticei* infective larvae reared in the laboratory<sup>2</sup>. Infective larvae were counted individually in five samples each in 4 range groups, covering the range 0-50, 100-200, 300-500, and 800-1200 larvae respectively. In each sample, the larvae were allowed to remain for half an hour on 10g parasite-free normal faecal pellets. The material was then allowed to remain immersed in warm water (40°C) in Baermann funnel for two days. At 24 and 48 hours interval, 25 ml of water was drawn off from the bottom and larvae present in the sediment and washing of the pipettes and tubes were counted microscopically.

The percentage recovery of the infective larvae from each sample ranged from 50.0 to 66.3%. An analysis of variance was carried out on percentage recovery after arcsin transformation of the percentage to stabilise the variance. The result:  $p < 0.05$  suggests that the recovery efficiency of the technique is independent of the numbers of infective larvae present within the range tested.

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Massey University,  
Palmerston North,  
New Zealand, September 30, 1974.

\* Present address: Research Officer, Livestock Research Station, Patna 800014, Bihar, India.

1. Soulsby, E. J. L., *Helminths, Arthropods and Protozoa of Domesticated Animals*. Bailliere Tindall and Cassell, London, 1968, p. 791.
2. Ahluwalia, J. S., "Studies on *Cooperia curticei* (Ransom, 1907), a nematode parasite of sheep," *Ph.D. Thesis*, Massey University, New Zealand, 1970.

### *Albizzia lebbek*—A New Host for Citrus White Fly (*Dialeurodes citri* Ashmead)

During July 1974 a large number of adults as well as nymphs of citrus white fly (*Dialeurodes citri* Ashmead) were found feeding on small seedlings of *Albizzia lebbek* which is grown as wind

break near the citrus orchards at Punjab Agricultural University, Ludhiana. Nature of damage was similar to that reported on citrus by Pruthi and Batra<sup>1</sup>.

The pest has been reported to feed on 42 different plant hosts which include different species of citrus and deciduous plants by Saini<sup>2</sup> and *A. lebbek* appears to be a new host for *D. citri*.

Department of Horticulture. S. K. KAPOOR.  
Punjab Agricultural University. S. P. KAPUR.  
Ludhiana, October 7, 1974. S. S. CHEEMA.

1. Pruthi, H. S. and Batra, H. N., *Misc. Bull. Imp. Coun. Agric. Res. India*, 1938, No. 19, 22.
2. Saini, B. S., "Bionomics and control of *Dialeurodes citri* (Riby and Howard) (Homoptera: Aleyrodidae)," *M.Sc. Thesis*, PAU, Ludhiana, 1964.

### Brinjal as a New Host of Yellow Hairy Caterpillar, *Psalis pennatula* (F.) (Lymantriidae: Lepidoptera)

During October, 1974 the younger stage larvae of hairy caterpillar were observed feeding on brinjal crop at the Entomological Farm, Punjab Agricultural University, Ludhiana. The larvae were collected and reared in the laboratory on brinjal leaves. The adults which emerged were identified as *Psalis pennatula* (F.). The survey of the literature revealed its earlier record on paddy, *Cholam*, ragi, sugarcane, grasses, cowpea and truck. Thus it appears to be the first record of this insect on brinjal.

The authors are grateful to Prof. O. S. Bhandra for providing the facilities and to Dr. G. S. Sandhu, Entomologist (Research), for identification of the insect.

Department of Entomology, DARSHAN SINGH.  
Punjab Agricultural University, M. RAMZAN.  
Ludhiana (Pb.), December 16, 1974.

### ANNOUNCEMENTS

#### International Symposium on Reproductive Physiology of Invertebrates

The above symposium will be held on September 10-12, 1975 at Calicut University, Kerala (India). The last date for registration is May 1, 1975.

For details please contact: Dr. K. G. Adiyodi, Convener, Department of Zoology, Calicut University, Kerala 673635.

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L. K. SINGH AND K. N. SINGH

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(MS. 50)

The temperature dependence of light output of the ZnO-MnO<sub>2</sub> phosphor has been studied. The phosphor thus obtained exhibits peak light output at 200°C. The intensity of the emission is maximum at 200°C. The temperature dependence of the intensity of the emission is studied. The relationship between the intensity of the emission and the temperature is studied.

### INTRODUCTION

THE temperature dependence of electroluminescent brightness is an important characteristic of Deslaur effect and is of much help for understanding the nature of trap levels which exert remarkable influence on the emission characteristics of electroluminescent phosphors. Deslaur studied the behaviour of ZnO, ZnS type phosphors and observed that the threshold voltage of excitation falls as the temperature is lowered. Later works due to Gobrecht, Roberts, Hults, Neumark, Matz, Thornton and Harker in this direction are also remarkable. Oskot<sup>1</sup> has observed that the emission characteristics are mainly concentrated in yellow-orange region.

### EXPERIMENTAL

The zinc oxide is activated with trace quantities (10<sup>-3</sup> g/g) of Mn and fired in nitrogen atmosphere around 1000°C. The phosphor thus obtained shows yellow-orange electroluminescence. For temperature studies, permanent type of electroluminescent cells were formed by dispersing the electroluminescent in Anadite between two conductive but transparent plane parallel mica sheets. The cell so formed was excited at different voltages and frequencies with the help of an audio oscillator cum wide band amplifier unit and the light output was recorded with photomultiplier and ultrasensitive microammeter assembly.

Such a cell was mounted over a copper sheet fitted at the end of a copper rod, about 10" in length and 1" in diameter. The desired temperature was achieved by cooling the rod with the liquid air or by heating it with the help of a cylindrical replaceable heater. The temperature was measured with a copper constantan thermocouple.

### RESULTS AND DISCUSSION

As usual with the electroluminescent of the ZnS-family, the Mn activation in ZnO also gives

the emission in the yellow-orange region. The light output of the phosphor is maximum at 200°C. The intensity of the emission is maximum at 200°C. The temperature dependence of the intensity of the emission is studied. The relationship between the intensity of the emission and the temperature is studied.

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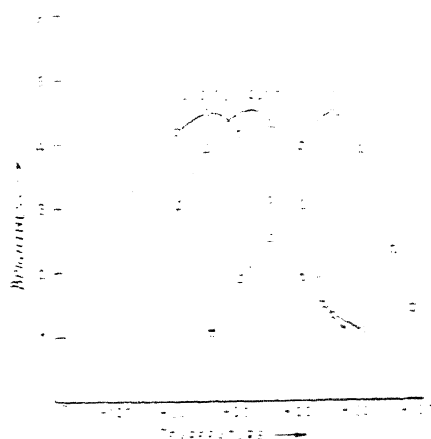


Fig. 1. Brightness-Temperature curves for the electroluminescent ZnO-MnO<sub>2</sub> phosphor at different exciting frequencies and 100 V, 100 Hz, 200 Hz.

This is due to the fact that the rate of recombination of the available time for the recombination of the electrons is decreased, and the energy of the electrons is not enough to make the traps empty. The shift is about 50°C when the electric frequency changes from 200 Hz to 50 Hz. These observations are found in accordance with the observations of No. secondary peaks of electroluminescence observed previously and the effect of electric field.

The temperature dependence of the intensity of the emission is studied. The relationship between the intensity of the emission and the temperature is studied.

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# Test of Baermann Technique for the Recovery of Infective Larvae of *Cooperia curticei* from Faecal Pellets

In ecological and epidemiological studies of strongyle nematodosis the recovery of the infective larvae from cultures of faecal pellets may be necessary. Infective larvae are generally recovered from faecal pellets by Baermann technique but the efficiency of this technique may depend on the bulk and physical conditions of the faecal culture. A test of this technique was done with known number of *Cooperia curticei* infective larvae reared in the laboratory<sup>1</sup>. Infective larvae were counted individually in five samples each in 4 range groups, covering the range 0-20, 100-200, 300-500, and 800-1200 larvae respectively. In each sample, the larvae were allowed to remain for half an hour on 10g parasite-free normal faecal pellets. The material was then allowed to remain immersed in warm water (40°C) in Baermann funnel for two days. At 24 and 48 hours interval, 25 ml of water was drawn off from the bottom and larvae present in the sediment and washing of the pipettes and tubes were counted microscopically.

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1. Soulsby, E. J. L. *Helminths, Arthropods and Protozoa of Domesticated Animals*. Baillière Tindall and Cassell, London, 1968, p. 791.
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The pest has been reported to feed on 42 different plant hosts which include different species of citrus and deciduous plants by Saini<sup>2</sup> and *A. lebbek* appears to be a new host for *D. citri*.

Department of Horticulture, S. K. KAPOOR,  
Punjab Agricultural University, S. P. KAPUR,  
Ludhiana, October 7, 1974. S. S. CHEEMA.

1. Pruthi, H. S., and Batra, H. N., *Misc. Bull. Imp. Coun. Agric. Res. India*, 1938, No. 19, 22.
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The authors are grateful to Prof. O. S. Bhandra for providing the facilities and to Dr. G. S. Sandhu, Entomologist (Research), for identification of the insect.

Department of Entomology, DARSHAN SINGH,  
Punjab Agricultural University, M. RAMZAN,  
Ludhiana (Pb.), December 16, 1974.

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For details please contact: Dr. K. G. Adiyodi, Convener, Department of Zoology, Calicut University, Kerala 673635.

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### Test of Baermann Technique for the Recovery of Infective Larvae of *Cooperia curticei* from Faecal Pellets

In ecological and epidemiological studies of strongylate nematodes the recovery of the infective larvae from cultures of faecal pellets may be necessary. Infective larvae are generally recovered from faecal pellets by Baermann technique<sup>1</sup> but the efficiency of this technique may depend on the bulk and physical conditions of the faecal culture. A test of this technique was done with known number of *Cooperia curticei* infective larvae reared in the laboratory<sup>2</sup>. Infective larvae were counted individually in five samples each in 4 range groups, covering the range 0-50, 100-200, 300-500, and 800-1200 larvae respectively. In each sample, the larvae were allowed to remain for half an hour on 10 g parasite-free normal faecal pellets. The material was then allowed to remain immersed in warm water (40°C) in Baermann funnel for two days. At 24 and 48 hours interval, 25 ml of water was drawn off from the bottom and larvae present in the sediment and washing of the pipettes and tubes were counted microscopically.

The percentage recovery of the infective larvae from each sample ranged from 50.0 to 66.3%. An analysis of variance was carried out on percentage recovery after arcsin transformation of the percentage to stabilise the variance. The result  $p < 0.05$  suggests that the recovery efficiency of the technique is independent of the numbers of infective larvae present within the range tested.

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# TEMPERATURE DEPENDENCE OF ELECTROLUMINESCENCE OF ZnO : Mn PHOSPHORS

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## ABSTRACT

The temperature dependence of the electroluminescence around 5900 Å. of ZnO : Mn phosphor have been investigated. The brightness-temperature curves for Mn-emission exhibit peak shift with the rise of excitation frequency, but with rise in voltage only over all intensity is increased. The temperature dependence of threshold excitation frequency obeys the relationship  $\log \nu \text{ (Hz)} = c_1 - c_2/T$ .

## INTRODUCTION

THE temperature dependence of electroluminescent brightness is an important characteristic of Destriau effect and is of much help for understanding the nature of trap levels which exert remarkable influence on the emission characteristics of electroluminophors. Destriau<sup>1</sup> studied the behaviour of ZnO, ZnS type phosphors and observed that the threshold voltage of excitation falls as the temperature is lowered. Later works due to Gobrecht<sup>2</sup>, Roberts<sup>3</sup>, Halsted<sup>4</sup>, Neumark<sup>5</sup>, Mattler<sup>6</sup>, Thornton<sup>7</sup> and Haake<sup>8</sup> in this direction are also remarkable. Osiko<sup>9</sup> has observed that the emission characteristics are mainly concentrated in yellow-orange region.

## EXPERIMENTAL

The zinc oxide is activated with trace quantities ( $10^{-4}$  g/g) of Mn and fired in nitrogen atmosphere around 1000°C. The phosphor thus obtained shows yellow-orange electroluminescence. For temperature studies, permanent type of electroluminescent cells were formed by dispersing the electroluminophor in Araldite between two conductive but transparent plane parallel mica sheets. The cell so formed was excited at different voltages and frequencies with the help of an audio oscillator cum wide band amplifier unit and the light output was recorded with photomultiplier and ultrasensitive microammeter assembly.

Such a cell was mounted over a copper sheet fitted at the end of a copper rod, about 10" in length and 1" in diameter. The desired temperature was achieved by cooling the rod with the liquid air or by heating it with the help of a cylindrical replaceable heater. The temperature was measured with a copper constantan thermocouple.

## RESULTS AND DISCUSSION

As usual with the electroluminophors of the ZnS-family, the Mn activation in ZnO also gives

the emission in yellow-orange region. The emission peak is observed around 5900 Å. We further notice that as the temperature rises, the intensity of yellow-orange emission of Mn increases fast with very little shift in peak position. As the temperature is further increased there is a fall in the intensity due to quenching effect.

## FREQUENCY DEPENDENCE

The brightness *versus* temperature curves exhibit that as the excitation frequency is increased, the emission peak shifts to high temperature (Fig. 1).

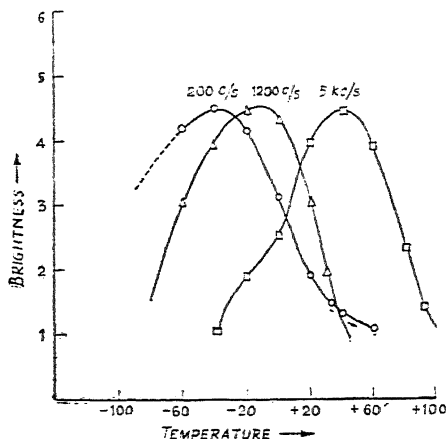


FIG. 1. Brightness vs. Temperature curves for the electro-luminophor ZnO : Mn excited by different exciting frequencies at fixed voltage near 600 V.

This is due to the fact that with increased frequency the available time for the exhaustion of the trapped electrons is decreased, requiring higher thermal energy to make the traps empty<sup>5</sup>. This shift is about 80°C when the excitation frequency changes from 200 c/s to 5 kc/s. These observations are found in accordance with the well-known facts<sup>10,11</sup>. No secondary peaks on temperature scale are observed probably due to absence of deeper traps.

The temperature effect at different voltages of excitations were tried. Plots of  $\log \nu \text{ (Hz)}$  vs.

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1/T (Fig. 2) gave straight lines obeying the relationship<sup>12</sup>  $\log \nu \text{ (Hz)} = c_1 - c_2/T$ . The slope of these straight lines decreased with the rise of

peratures. This type of behaviour at low temperature may be correlated with the decrease in trap depth.

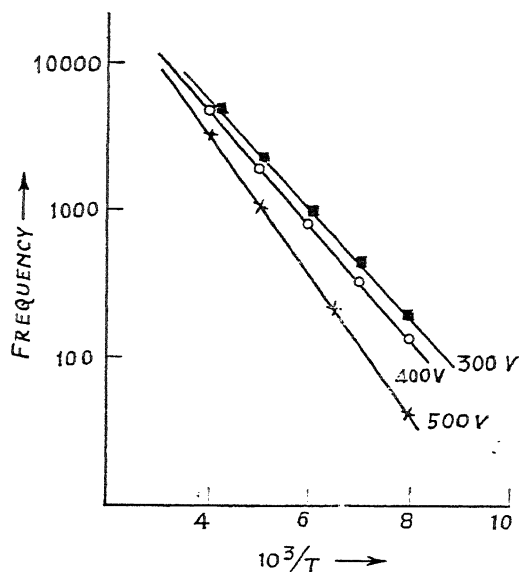


FIG. 2. Dependence of threshold frequencies of excitation with the temperature for ZnO : Mn/. excitation voltages, which means, higher threshold frequencies are required at the lower voltages for excitation.

**Voltage Dependence.**—Increase in voltage enhances the brightness (Fig. 3) at different temperatures but no peak shift is observed. This peak is found around 20°C when the excitation field frequency is kept at 1.5 kc/s. At 600 volts, the brightness is enhanced considerably at low tem-

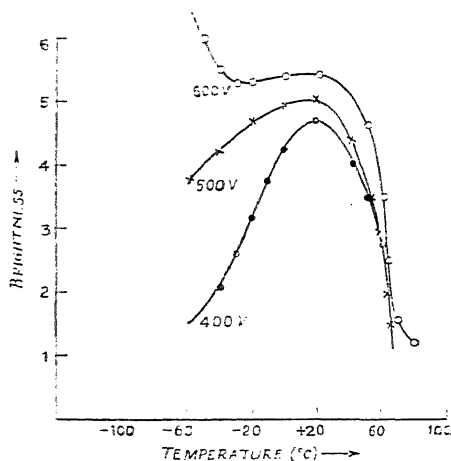


FIG. 3. Brightness vs Temperature curves for the electroluminescent ZnO : Mn/ excited by different a.c. voltages with fixed frequencies (1500 c/s) of field.

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## 2-HYDROXY-3,5-DICHLOROACETOPHENONE OXIME AS AN ANALYTICAL REAGENT : GRAVIMETRIC DETERMINATION OF COPPER, NICKEL AND COBALT

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### ABSTRACT

2-Hydroxy-3,5-Dichloroacetophenone Oxime (HDCAO), has been found to be a good reagent for gravimetric estimation of copper, nickel and cobalt and for their separation from other ions. The composition of the complexes is 1:2 as determined by modified continuous variation method.

OXIMES have been used as analytical reagents for the gravimetric as well as spectrophotometric determination of a number of metal ions<sup>1-3</sup>. In an earlier communication<sup>4</sup>, conductometric, potentiometric, micro-analysis and I.R. spectral

studies were reported for these metal complexes. The reagent has now been successfully employed for the gravimetric determination of copper, nickel and cobalt and their separation from a number of other ions.

# EXPERIMENTAL

2-Hydroxy-3,5-Dichloroacetophenone Oxime (HDCAO) was prepared as described earlier<sup>1</sup>. An ethanolic solution of the ligand was employed. Solutions of copper, nickel and cobalt salts were prepared from B.D.H. (A.R.) samples and standardized gravimetrically. Solutions of other ions were prepared from Reagent Grade samples. Systronic pH meter type 322 was used for pH measurement.

**Determination of Copper.**—The thiocyanate method suffers from the disadvantage of a low conversion factor and takes a long time (~6 hr) for complete precipitation. Salicylaldoxime and *o*-hydroxyacetophenone oxime complexes are somewhat soluble in ethanol. As a consequence, excess of reagent which gets precipitated is difficult to wash. But the complexes of HDCAO with copper, nickel and cobalt are insoluble in 60% ethanol and the excess of reagent that is precipitated can easily be washed. The pH ranges for complete precipitation and also separation of copper from nickel and cobalt are wider as compared to salicylaldoxime and *o*-hydroxy acetophenone oxime.

The pH of an aqueous solution (~100 ml) containing about 10 mg of copper was adjusted to known value using acetic acid and ammonia buffer. The solution was heated to 60–70°C and treated with an ethanolic solution of HDCAO (0.5%) dropwise (about twice the theoretical value) with constant stirring. The buff coloured precipitate was digested on a water bath for about 30 min. It was filtered while hot, through a sintered glass crucible (G-4) and washed with hot water. The precipitate was finally washed several times with 50% ethanol and was dried at 100–120°C to constant weight. It has been found that copper can be quantitatively determined in the pH range 3.0–10.0 although the precipitation starts at pH 2.0. The conversion factor (metal/metal complex) is 0.1267.

**Study of Interference.**—By suitable adjustment of pH, excess of the following cations (five to ten times) could be tolerated: Fe (II), Fe (III), Bi (III), Sb (III), As (III) (at pH 3.0); Ni (II), Co (II), Zn (II), Cd (II), Mo (VI), Hg (II), Mn (II), Mg (II), W (VI), UO<sub>2</sub> (II) (at pH 4.0). About 10–100 times of molar excess of several anions could be tolerated at pH 4.0 (PO<sub>4</sub><sup>3-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, F<sup>-</sup>, B<sub>4</sub>O<sub>7</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and tartrate).

**Determination of Copper in Alloys.**—A known amount of the alloy (brass, aluminium-bronze, manganese-bronze) was dissolved in conc. HNO<sub>3</sub>. The excess of acid was evaporated. The stannic oxide was removed by filtration. Copper was determined in the filtrate at pH 3.0, as described

earlier. The other ions did not interfere. It has been found that the percentage of copper obtained in different alloys using this method agrees with the reported values within experimental errors.

**Determination of Nickel.**—It has been found that HDCAO can precipitate (green) nickel quantitatively in about 100 ml solution containing 8 mg nickel ion in the pH range 5.0–9.0 using acetic acid and ammonia buffer for adjusting the pH. The experimental procedure for the precipitation of nickel was the same as described in the case of copper. The conversion factor is 0.1182.

**Study of Interference.**—Wherever possible, the interfering ions have been masked with suitable masking agents, e.g., Fe (III), Sb (III), Bi (III) by tartaric acid (1 gm). In some cases, the interference has been removed by changing the pH of the mixed solution, e.g., cations like Zn (II), Mn (II) (at pH 5.5); Cd (II), UO<sub>2</sub> (II), Mg (II), Mo (VI), W (VI), Hg (II) (at pH 6.0); Fe (III), Sb (III), Bi (III), As (III) (at pH 7.5) (from 5–10 times excess) and sufficiently large excess of anions like PO<sub>4</sub><sup>3-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, F<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, B<sub>4</sub>O<sub>7</sub><sup>2-</sup> and tartrate (at pH 6.0).

**Determination of nickel in alloys.**—A known amount of the alloy (aluminium-bronze, high tensile brass and coin) was dissolved in conc. HNO<sub>3</sub> and the tin removed as SnO<sub>2</sub>. The copper in the filtrate was first determined at pH 3.0 and the nickel was then estimated by raising the pH of the filtrate to 8.0.

**Determination of Cobalt.**—HDCAO has also been found to precipitate (orange) cobalt quantitatively. The pH of cobalt solution about 100 ml containing 9 mg of cobalt was adjusted to 8–9 using acetate-borax buffer. The experimental procedure for the precipitation of cobalt was the same as described in the case of copper. The precipitate was washed with hot water and finally with 25% alcohol. The conversion factor was 0.1186.

**Study of Interference.**—The cations Zn (II), Mn (II), Mg (II), Cd (II), As (III), Mo (VI), W (VI) (five to seven times) and excess of the anions Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and tartrate do not interfere in the determination of cobalt at pH 7.5.

From the solution, containing both copper and cobalt, the copper was precipitated as described earlier at pH 3.0 and from the filtrate cobalt was precipitated by the addition of more reagent and adjusting pH to 7.5.

**Accuracy of the estimation.**—Copper, nickel (5–50 mg) could be estimated with an accuracy of ±0.3% while in the case of cobalt (10–50 mg) the percentage error was ±0.4%. At lower concentrations of the metals (3–5 mg) the percentage error was about 5% higher.

The composition of the complexes as determined above and also by using modified continuous variation method<sup>6</sup> is 1:2.

#### ACKNOWLEDGEMENT

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## ZIRCONIUM(IV) COMPLEXES WITH MULTIDENTATE SCHIFF BASES

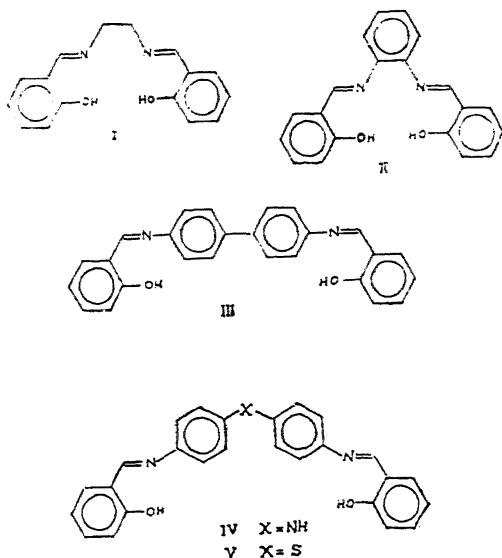
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#### INTRODUCTION

IN the previous papers we showed that zirconium exhibits coordination numbers five and eight when complexed with bidentate and tetradentate Schiff bases<sup>1-3</sup>. Macarovici *et al.*<sup>4</sup> have demonstrated that zirconium forms complexes of coordination number six with Schiff bases derived from benzidine. There is not much information on the zirconium (IV) complexes with multidentate Schiff bases<sup>4-5</sup>.

In this paper we report the synthesis and spectral study of a few zirconium (IV) complexes with multidentate Schiff bases shown below.



#### EXPERIMENTAL

Zirconyl chloride used for preparing the complexes was of Fluka make. *p,p'*-Diaminodiphenyl

sulphide was prepared by the known method<sup>6</sup>. Schiff bases were prepared by Biradar's method<sup>7</sup>.

Zirconyl chloride in methanol was treated with a slight excess of Schiff base in the same solvent. This mixture was refluxed for 2-3 hours on water bath. The complex separated out was filtered and washed free from the reagent with methanol, then with ether and dried in vacuum. The complexes were analysed for zirconium, nitrogen and chloride contents<sup>8</sup>.

#### RESULTS AND DISCUSSION

It is evident from the elemental analysis (Table I) that these bases form the complexes of 1:1 stoichiometry.

**Infrared Spectra.**—Infrared spectra of the Schiff bases and complexes in Nujol mulls have been taken with a Perkin Elmer 137 B spectrometer. The strong band in the region 1618-1613 cm<sup>-1</sup> attributable to the C=N stretching is found in the region 1640-1626 cm<sup>-1</sup> in these zirconium (IV) complexes. The shift towards higher frequency indicates coordination through the azomethine nitrogen.

The broad weak band in the region 2800-2700 cm<sup>-1</sup> due to the intramolecular hydrogen bonded OH is not observed in the complexes and the band due to the phenolic C-O in the region 1280-1270 cm<sup>-1</sup> is found around 1300 cm<sup>-1</sup>. These observations show that the hydroxy groups of the base are involved in the bond formation. Analogous observations have been made by Kovacic<sup>9a</sup> in copper (II) Schiff base complexes and Marvel *et al.*<sup>9b</sup>.

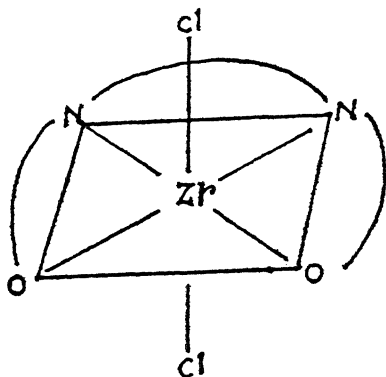
It has been established that when a compound contains M=O, the infrared spectrum shows a narrow intense band in the region 1100-800 cm<sup>-1</sup> whereas a broad intense band in the corresponding region indicates the presence of a polymeric M-O-M

TABLE I  
Elemental analysis of the complexes

| Lig. No. | Comp. No. | Empirical formula   | % Zr          | % N         | % Cl          |
|----------|-----------|---|---------------|-------------|---------------|
| I        | 1         | (C <sub>16</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> ) ZrCl <sub>2</sub>  | 20.88 (21.30) | 6.13 (6.54) | 15.85 (16.54) |
| II       | 2         | (C <sub>20</sub> H <sub>13</sub> N <sub>2</sub> O <sub>2</sub> ) ZrCl <sub>2</sub>  | 19.20 (19.60) | 6.00 (6.42) | 15.02 (15.26) |
| III      | 3         | (C <sub>20</sub> H <sub>13</sub> N <sub>2</sub> O <sub>2</sub> ) ZrCl <sub>2</sub>  | 16.20 (16.52) | 5.02 (5.07) | 12.00 (12.86) |
| IV       | 4         | (C <sub>20</sub> H <sub>13</sub> N <sub>2</sub> O <sub>2</sub> ) ZrCl <sub>2</sub>  | 16.55 (16.08) | 7.36 (7.40) | 12.80 (12.51) |
| V        | 5         | (C <sub>20</sub> H <sub>13</sub> SN <sub>2</sub> O <sub>2</sub> ) ZrCl <sub>2</sub> | 15.40 (15.65) | 5.07 (4.79) | 12.03 (12.10) |

chain<sup>10</sup>. Patel and coworkers<sup>11,12</sup> have assigned a narrow intense band around 980 cm<sup>-1</sup> to Zr=O stretch in zirconium complexes. Biradar *et al.*<sup>3</sup> have assigned the band around 917 cm<sup>-1</sup> to Zr=O stretch in zirconyl (II) Schiff base complexes. These observations and the comparative study of the infrared spectra of the Schiff bases and the complexes suggest that these zirconium complexes do not contain either Zr=O or Zr-O-Zr moiety in them.

The base I is sterically favourable and can encompass the metal ion strainlessly<sup>12</sup>, preferring *cis* configuration<sup>13</sup>. The base II is a more rigid tetradentate as the CH<sub>2</sub>-CH<sub>2</sub> is replaced by a phenyl ring. This too prefers to have *cis* configuration. Both the complexes are non-electrolytes in DMF and have a coordination number of six. It may therefore be assumed that the two chlorine atoms occupy the remaining *trans* positions of the octahedron as shown below:



Though the complex 3 with base III has been prepared by a different method it has the same composition as that reported by Macarovici *et al.*<sup>4</sup>. On steric grounds and in view of previous observations<sup>14,15,16</sup> one can assign the dimeric structure to this complex. The dimeric nature of the complex could not be substantiated by the molecular weight determination as this complex is not soluble in common organic solvents.

The bases IV to V can be compared with the Schiff bases formed by condensing salicylaldehyde with diamino diethyl sulphide and diaminodiethyl amine<sup>17</sup>. These bases (IV and V) are bulkier due to the *p*-substituted phenyl groups and involve comparatively much strain. The construction of the models shows that the dimeric structure involves comparatively less strain than the monomeric structure. However, the coordination number of zirconium in these complexes remains six as neither NH of the base IV nor S of the base V takes part in the coordinate bond formation. It has been observed by Bailar and Sarma<sup>18</sup> that coordination of the NH group increases the multiplicity of it and splits the band due to NH. The non-splitting of the NH band at 3300 cm<sup>-1</sup> in the complex 4 indicates that -NH- of the base IV has not taken part in coordinate bond formation. In analogy with the base IV it can be said that -S- of the base V cannot take part in the coordination. Analogous observations have also been reported by Taylor and Coleman<sup>16</sup>. All these observations show that the bases IV and V show tetradentate behaviour.

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## NATIONAL CONFERENCE ON CRYSTALLOGRAPHY

A NATIONAL Conference on Crystallography, sponsored by the National Committee for Crystallography (Indian National Science Academy) was held at the Indian Institute of Science, Bangalore, from 26 to 29 December, 1974. A special Symposium on the "Structure and Conformation of Biological Molecules" and a Workshop on Crystallography were also arranged along with the Conference with financial support from the Council of Scientific and Industrial Research and the University Grants Commission respectively. The Conference, along with the Symposium and the Workshop, was organized and conducted by a Local Organizing Committee headed by Professor G. N. Ramachandran and consisting of senior crystallographers from the Indian Institute of Science, the National Aeronautical Laboratory and the Raman Research Institute.

The Conference was attended by about 130 crystallographers from different parts of India. In addition, three distinguished scientists from abroad, namely, Professor Alexander Rich of the Massachusetts Institute of Technology, Professor Walter Kauzmann of the Princeton University and Professor Henry Sobell of the Rochester University, also participated in the Conference.

The Conference, the Symposium and the Workshop were inaugurated by Professor Alexander Rich on the 26th at a function presided over by Professor S. Bhagavantam. In his inaugural address, Professor Rich described his celebrated work on the three-dimensional structure of yeast phenylalanine transfer RNA. He described the crystallographic techniques employed in the analysis and explained the three-dimensional architecture of the transfer RNA molecule.

### The Conference on Crystallography

The academic programme of the Conference (exclusive of the Symposium) consisted of four

invited talks and the presentation of about a hundred contributed papers. In his invited talk on crystal growth, Professor A. R. Patel described the reacted flux and gel methods of crystal growth developed in his laboratory at Anand. Dr. R. Chidambaram, in his talk, reviewed the evolution of single crystal diffractometers over the last decade and explained the salient features of the computer controlled diffractometer now being developed in the Bhabha Atomic Research Centre. Dr. P. Rama Rao presented a review of defects in metallic structures and their importance in understanding the strength and behaviour of metals and alloys. Dr. H. Manohar in his talk discussed the structural basis of topotactic reactions in crystals and described some examples of diffraction studies in this area. The contributed papers dealt with theoretical crystallography, the methods of structure determination, the x-ray analysis of organic and inorganic compounds, crystal defects and crystal growth, instrumentation, phase transitions and computer programming.

### The Symposium on the Structure and Conformation of Biological Molecules

The highlight of the Symposium on the "Structure and Conformation of Biological Molecules" was a talk by Professor H. M. Sobell on the x-ray visualization of drug-nucleic acid interactions. Professor Sobell demonstrated with the aid of molecular models and packing diagrams how plausible models for DNA-actinomycin and DNA-ethidium bromide interactions could be proposed on the basis of the x-ray structure analysis of model compounds. In another invited talk, Professor V. Sasisekharan discussed the importance of the effect of furanose ring puckering on the conformation of nucleic acid chains on the basis of energy calculations carried out by his group. The talk of Dr. Girish Govil was concerned with the organization of phospho-

lipids in biomembranes. The contributed papers submitted to the Symposium dealt with single crystal x-ray structure analysis of biologically important molecules, theoretical studies on the conformation of biomolecules, conformational studies in solution and enzyme crystallization.

#### The Workshop on Crystallography

The programme of the Workshop on Crystallography, held along with the Conference, consisted mainly of two evening lectures, and a one-day session of lectures and discussions on December 29. In his evening lecture on "Some problems in our understanding of protein structure", Professor W. Kauzmann showed how the information obtained from protein crystallography could be used in the study of the physical chemistry of proteins. Professor Sobell gave the other evening talk entitled "The stereochemistry of DNA strand equivalence in genetic recombination and its implication for models of recombination". The programme on

December 29 included two lecture-and-discussion sessions, one on computational problems in crystallography and the other on the direct methods in crystallography, which were led by Dr. A. Sequeira and Dr. K. Venkatesan respectively. Dr. S. Ramaseshan, in his talk on "Anomalous scattering of x-rays, neutrons and electrons", discussed the new developments in this area and reported the highlights of the recent International Conference on Anomalous Scattering held at Madrid. The other talks in the Workshop consisted of one by Dr. M. Vijayan on "Macromolecular crystallography" and another by Dr. C. Ramakrishnan on "X-ray diffraction by helical structures". In addition, the programme of the Workshop included a discussion on the x-ray diffraction equipments available in India. Representatives of some Indian manufacturers of x-ray equipments also participated in this discussion.

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I, S. R. S. Sastry, hereby declare that the particulars given above are true to the best of my knowledge and belief.

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## LETTERS TO THE EDITOR

CRYSTAL STRUCTURE OF LEAD ACETATE  
TRIHYDRATE

CRYSTAL of lead acetate trihydrate, commonly known as sugar of lead, are obtained by the gradual evaporation of a saturated solution of the substance. The morphological characteristics of the crystals agree well with those given by Gröth<sup>1</sup>. The crystals are monoclinic with the following cell dimensions:  $a = 15.8$ ,  $b = 7.31$ ,  $c = 9.07$  Å,  $\beta = 109.8^\circ$ . The density of the substance was measured using flotation technique with a liquid mixture of bromoform and carbon tetrachloride. There are four molecules in the unit cell. Systematic absences indicate that the space group can be either  $C2$ ,  $Cm$  or  $C2/m$ .

Crystal samples used in X-ray studies were coated with collodion to prevent loss of water of hydration. There is also pronounced radiation damage on exposure to X-rays necessitating the use of different specimens for different layers in the collection of the intensity data. Three-dimensional intensity data ( $hkl$ ,  $K = 0$  to 6,  $hkl$ ,  $L = 0$  to 2) were collected using  $Cu K\alpha$  radiation by the multiple film equi-inclination technique. Spot-shape, Lorentz-polarization and absorption corrections were applied in the usual way. Using the projection data, Patterson syntheses were computed and the heavy atom, lead, was located at  $(.33, 0, .09)$ . A three-dimensional Fourier and difference-Fourier syntheses based on the phases of lead atom revealed the light atoms unambiguously. With anisotropic thermal parameters for lead and isotropic thermal parameters for the rest of the atoms, least-squares refinement was carried out using the LALS program on the IBM 370/155 computer at the Indian Institute of Technology, Madras. For 796 observed reflections, the residual index at present is 0.13 in the space group  $C2/m$  whereas 0.21 with the heavy atom alone. The crystal data and the atomic coordinates are presented in Table I.

Preliminary dielectric measurements made on powder specimens indicate that the crystal can possibly be ferroelectric upto its melting point of  $75^\circ C$ . The reported piezoelectricity<sup>2</sup> and pyroelectricity<sup>3</sup> in literature are, perhaps, attributable to this crystal. In recent EPR studies<sup>4</sup> also, it is mentioned, without stating the reason that the noncentric space group  $C2$  is favoured. But the departure from the present structure based on the centric space group will be very slight.

TABLE I

*Crystal data and fractional atomic coordinates for lead acetate trihydrate*

$$a = 15.8 \text{ Å}, \quad b = 7.31 \text{ Å}, \quad c = 9.07 \text{ Å}, \quad \beta = 109.8^\circ, \\ V = 985.7 \text{ Å}^3, \quad \rho_{\text{obs}} = 2.55 \text{ gm. cm}^{-3}, \\ \rho_{\text{calc}} = 2.554 \text{ gm. cm}^{-3}, \quad \text{F.W.} = 379.5 \text{ gm}, \quad Z = 4, \\ \mu (\lambda = 1.5418 \text{ Å}) = 346.6 \text{ cm}^{-1}.$$

| Atom  | x      | y     | z      |
|-------|--------|-------|--------|
| Pb    | 0.3314 | 0.000 | 0.0867 |
| O (1) | 0.232  | 0.000 | 0.226  |
| O (2) | 0.358  | 0.000 | 0.408  |
| O (3) | 0.265  | 0.373 | 0.099  |
| O (4) | 0.068  | 0.000 | 0.116  |
| O (5) | 0.098  | 0.500 | 0.097  |
| O (6) | 0.026  | 0.500 | 0.287  |
| C (1) | 0.277  | 0.000 | 0.369  |
| C (2) | 0.229  | 0.000 | 0.489  |
| C (3) | 0.317  | 0.500 | 0.162  |
| C (4) | 0.411  | 0.500 | 0.277  |

The first three oxygen atoms belong to the acetate groups and the latter three belong to the water molecules.

The lead atom is coordinated to eight oxygen atoms (the Pb-O distances varying from 2.31 to 3.10 Å) out of which two belong to the water molecules. It is interesting to note that both the oxygen atoms of the acetate groups coordinate with the lead. The geometry of the acetate groups resembles that found in similar structures. All the water molecules and acetate oxygens take part in a complicated network of hydrogen bonding. A noticeable feature of this hydrogen bonding is that the bonds are perpendicular to the  $b$ -axis.

Further refinement is under progress. Efforts will be made to refine the structure in the noncentric space group  $C2$  also.

The authors are thankful to Prof. K. S. Chandrasekaran for his keen interest in this problem and one of them (R. K. R) is thankful to the Council

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### DEUTERIUM EFFECT ON LIFETIME OF URANYL COMPLEXES IN SOLUTIONS

In solutions, uranyl ion ( $\text{UO}_2^{++}$ ) forms different complexes, viz.,  $\text{UO}_2^{++} (6\text{H}_2\text{O})$ , anionic complexes, e.g.,  $\text{UO}_2(\text{NO}_3)_2$  or  $\text{UO}_2\text{SO}_4$  and hydrolysed species  $\text{UO}_2(\text{UO}_3)_n$ , etc.<sup>1</sup> In the present study we have measured lifetimes of different uranyl complexes in various protonated solutions and their deuterated counterparts at 80° K. Deuteration has been accomplished in such a manner that for both the protonated and deuterated solutions, the conditions of pH, concentration and strength of the solvent were same except for the replacement H atoms of the solvent by D. Lifetimes of these solutions (Uranyl ion

concentration 0.1 M) obtained by exciting them at 3650 Å with the help of a pulse halfwidth 5 nsec, are given in Table I.

It is seen from Table I that nearly in all uranyl solutions, the lifetimes increase in deuterated solvents; but large deuteration effect is observed for  $\text{UO}_2^{++} (6\text{H}_2\text{O})$  complex, present in aqueous solutions of uranyl nitrate and perchlorate  $\tau_D/\tau_H \sim 6$ , whereas, no prominent increase is observed in solutions containing uranyl complexes like  $\text{UO}_2(\text{NO}_3)_2$ , etc., or in hydrolysed complexes  $\text{UO}_2(\text{NO}_3)_n$   $\tau_D/\tau_H \sim 1.2$ .

The effect of solvent deuteration, upon lifetimes, comes from inhibition of radiationless transitions which occur due to the exchange of electronic energy from the emitting state to the high frequency O-H vibrations. The overlap integral, determining the rate of radiationless transitions, becomes smaller for relatively lower frequency O-D vibrations resulting in an increase in the observed lifetime<sup>2-4</sup>. Large deuteration effect is expected to occur in the systems for which the rate for radiationless transitions is comparable with the radiative transition rate. Our results for uranyl complexes show that for  $\text{UO}_2^{++} (6\text{H}_2\text{O})$  complex, the non-radiative transitions are more pronounced in comparison to other uranyl complexes. Complexation as well as hydrolysis reduces the radiationless transition probability. Little effect of deuteration in acidic solutions suggests that for the anionic complexes, the lifetimes are closer to their radiative lifetimes. This is evident as the quantum

TABLE I

| Uranyl Solutions prot./deut.  | Complex*  | $\tau_H$ $\mu$ sec. | $\tau_D$ $\mu$ sec. | $\tau_D/\tau_H$ |
|---|---|---------------------|---------------------|-----------------|
| $\text{UO}_2(\text{NO}_3)_2$ in 60% $\text{HNO}_3/\text{DNO}_3$                   | $\text{UO}_2(\text{NO}_3)_2$  | 800                 | 900                 | 1.12            |
| $\text{UO}_2\text{SO}_4$ 95% $\text{H}_2\text{SO}_4/\text{D}_2\text{SO}_4$        | $\text{UO}_2\text{SO}_4$  | 930                 | 1,120               | 1.20            |
| $\text{UO}_2(\text{NO}_3)_2$ in $\text{H}_2\text{O}/\text{D}_2\text{O}$           | $\text{UO}_2^{++} (6\text{H}_2\text{O})$                                | 250                 | 1,500               | 6.00            |
| $\text{UO}_2\text{SO}_4$ in $\text{H}_2\text{O}/\text{D}_2\text{O}$               | $(\text{UO}_3\text{OH})_2\text{SO}_4$ or<br>$\text{UO}_3\text{HSO}_4^-$ | 400                 | 720                 | 1.80            |
| $\text{UO}_2(\text{ClO}_4)_2$ in $\text{H}_2\text{O}$                             | $\text{UO}_2(6\text{H}_2\text{O})$                                      | 270                 | 1,600               | 5.70            |
| $\text{UO}_2(\text{CH}_3\text{COO})_2$ in $\text{H}_2\text{O}/\text{D}_2\text{O}$ | $\text{UO}_2\text{CH}_3\text{COO}^-$                                    | 340                 | 560                 | 1.60            |
| $\text{UO}_2(\text{NO}_3)_2$ in 0.1 M $\text{NaOH}/\text{NaOD}$                   | $\text{UO}_2(\text{UO}_3)_n^{--}$                                       | 70                  | 80                  | 1.14            |
| $\text{UO}_2(\text{NO}_3)_2$ in 0.01 M $\text{NaOH}/\text{NaOD}$                  | $\text{UO}_2\text{UO}_2^{--}$   | 220                 | 350                 | 1.60            |
| $\text{UO}_2(\text{NO}_3)_2$ in $\text{CH}_3\text{OH}/\text{CH}_3\text{OD}$       | $\text{UO}_2^{--}$<br>and<br>$\text{UO}_2\text{NO}_3^-$                 | 250<br>650          | 450<br>750          | 1.80<br>1.20    |

\* The complexes are in protonated solvents, in deuterated solvents H is replaced by D except for the ligand  $\text{CH}_3\text{COO}^-$ .

yield of uranyl sulphate in sulphuric acid is unity at 77°K.

The higher rate of non-radiative processes, in aqueous solutions of uranyl nitrate and perchlorate appears to be due to the presence of water molecules in the first coordination sphere of uranyl ion. In this case direct coupling between uranyl ion and water molecules facilitates non-radiative energy migration from excited uranyl ion to high frequency O-H vibrations. In acidic solutions, in the complexes like  $\text{UO}_2(\text{NO}_3)_2$  and  $\text{UO}_2\text{SO}_4$ , anions replace coordinated water molecules. Such shielding effect reduces the non-radiative dissipation of energy. The hydrolysed complexes are also shielded by coordination with  $\text{UO}_3$  and the results are similar to the anionic complexation. In the aqueous solutions of uranyl sulphate and acetate, uranyl ion is partially complexed and partially exposed to coordinate with water molecules, thereby showing somewhat greater deuteration effect than the complexed species.

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## COBALT (III) HETEROCHELATES CONTAINING NONPLANAR QUADRIDENTATE LIGANDS

COBALT (III) complexes containing nonplanar quadridentate Schiff base salen [where salen  $\text{H}_2 = \text{N}, \text{N}'$ -ethylenediaminebis (salicylideneimine)] and  $\beta$ -diketones have been the object of much work<sup>1-4</sup>. In this preliminary communication we describe the synthesis of several new cobalt (III) heterochelates containing quadridentate ligands saltn or salpn [where saltn  $\text{H}_2 = \text{N}, \text{N}'$ -trimethylenediaminebis (salicylideneimine), salpn  $\text{H}_2 = \text{N}, \text{N}'$ -propylenediaminebis (salicylideneimine)] and bidentate monobasic ligands like acetylacetone, 8-hydroxyquinoline, tropolone and acetoacetanilide.

$\text{Co}(\text{saltn})$  and  $\text{Co}(\text{salpn})$  were prepared according to the method of Bailes and Calvin<sup>5</sup>.

For the preparation of the mixed ligand complexes equimolar amounts  $\text{Co}(\text{saltn})$  or  $\text{Co}(\text{salpn})$  and the appropriate bidentate ligand in absolute alcohol were refluxed and filtered. The evaporation of the filtrate produced green precipitates of the complexes. The complexes were recrystallised from ethanol or 1:1 mixture of ethanol and chloroform. The acetoacetanilide complexes were recrystallised from acetone. The characterisation data of the complexes are presented in Table I.

The electrolytic conductance measurements of the complexes in methanol indicate non-electrolytic nature of the complexes. The complexes are diamagnetic as expected for  $d^6$  cobalt (III) complexes of octahedral symmetry. The electronic spectra of the complexes in chloroform exhibit two bands at around 16500 and 25000  $\text{cm}^{-1}$  characteristic of octahedral cobalt (III) complexes<sup>6</sup>. The first band at 16500  $\text{cm}^{-1}$  is assigned to the  ${}^1\text{A}_{1g} \rightarrow {}^1\text{T}_{1g}$  transition and the second band at 2500  $\text{cm}^{-1}$  is assigned to the metal ligand transitions coupled with the

TABLE I  
Characterisation data of cobalt (III) complexes<sup>a,b</sup>

| Complex                                     | % Co                        | % N         | Band I $\text{cm}^{-1}$ | Band II $\text{cm}^{-1}$ |
|---|-----------------------------|-------------|-------------------------|--------------------------|
| $\text{Co}(\text{saltn})(\text{acac})$      | Found: 13.7<br>Reqd.: 13.4  | 6.7<br>6.39 | 16830 (2.53)            | 26180 (3.83)             |
| $\text{Co}(\text{saltn})(\text{tropolone})$ | Found: 12.6<br>Reqd.: 12.83 | 5.7<br>6.09 | 16720 (2.51)            | 26250 (4.03)             |
| $\text{Co}(\text{saltn})(\text{acan})$      | Found: 11.9<br>Reqd.: 11.46 | 8.0<br>8.16 | 16670 (2.50)            | 26180 (3.80)             |
| $\text{Co}(\text{saltn})(\text{oxine})$     | Found: 11.9<br>Reqd.: 12.22 | 9.0<br>8.70 | ..                      | 24540 (3.98)             |
| $\text{Co}(\text{salpn})(\text{acac})$      | Found: 13.6<br>Reqd.: 13.47 | 6.5<br>6.39 | 16670 (2.55)            | 25510 (3.58)             |

TABLE I—Contd.

| Complex               | %Co                         | %N          | Band I $\text{cm}^{-1}$ | Band II $\text{cm}^{-1}$ |
|-----------------------|-----------------------------|-------------|-------------------------|--------------------------|
| Co(salpn) (tropolone) | Found: 12.3<br>Reqd.: 12.83 | 5.7<br>6.09 | 16900 (2.54)            | 25640 (4.09)             |
| Co(salpn) (acan)      | Found: 11.1<br>Reqd.: 11.46 | 8.4<br>8.16 | 16950 (2.50)            | 25510 (3.78)             |
| Co(salpn) (oxine)     | Found: 12.0<br>Reqd.: 12.22 | 9.0<br>8.70 | 17120 (2.60)            | 25250 (3.94)             |

<sup>a</sup> Figures in the parentheses indicate  $\log \epsilon$ .

<sup>b</sup> Abbreviations: acac = deprotonated anion of acetylacetone; acan = deprotonated anion of acetoacetonilide; oxine = deprotonated anion of 8-hydroxyquinoline.

$1A_{1g} \rightarrow {}^1T_{1g}$  transition. The *cis* spanning of the bidentate ligand in the complexes force the quadridentate ligands salpn and salpn to coordinate to cobalt (III) in a nonplanar twisted configuration similar to salen in Co(salen) ( $\beta$ -diketone)<sup>3</sup>.

The synthesis of mixed ligand complexes utilising other bidentate NO, NN and OO donor ligands and also the synthesis of Co(acen) (BB) (where acen  $\text{H}_2 = \text{N}, \text{N}'$ -ethylenediaminebis (acetylacetoneimine) and BB = bidentate ligand) are in progress.

The details of the work will be reported later.

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#### KINETICS OF REACTIONS OF TERTIARY AMINES WITH BENZYL HALIDES

Brown and Eldred<sup>1</sup> and Brown and Fried<sup>2</sup> have investigated quaternisation of a number of tertiary amines with alkyl halides. Baker and Nathan<sup>3</sup> have compared the reactions of pyridine and  $\alpha$ -picoline

with arylbromide and alkylbromides and the differences in reactivities have been attributed to differences in energy of activation. Solvent influences on certain quaternisation processes have also been studied by Padmanabhan and Anantakrishnan<sup>4</sup>. This note deals with the kinetics of reactions of pyridines, quinoline, isoquinoline and their derivatives with benzyl and substituted benzyl chlorides in nitrobenzene and nitrobenzene-ethanol mixture and the kinetic data have been used to compute the nucleophilicity parameters of the bases for the first time.

Kinetics has been followed by Volhard's method using A.R. grade substances mostly, except a few compounds which were purified using standard procedures.

The second order rate constants for the reactions of tertiary bases with benzyl halides have been calculated using the usual rate expression except in a few cases in nitrobenzene medium, where equilibrium was attained and in such cases the rate constant was evaluated using the modified rate expressions<sup>5</sup>.

As seen from Table I, plots of  $\log k$  for pyridines VS  $\text{Pka}$  are fairly linear except with  $\alpha$ -picoline. The order of reactivity is  $\gamma$ -picoline  $>$   $\beta$ -picoline  $>$  pyridine  $>$   $\alpha$ -picoline. Plots of  $\log k$  of bicyclic bases VS  $\text{Pka}$  shows considerable scatter probably due to want of accurate  $\text{Pka}$  values in the solvent system studied. Among the bicyclic bases, isoquinoline reacts faster than quinoline but the order is reversed when quinoline nucleus is substituted with a methyl group.

Among the benzyl chlorides, *p*-methyl benzyl chloride reacts fastest due to the electron releasing nature of the methyl substituent. The lower reactivity of chlorobenzyl chlorides can be attributed to the electron attracting nature of the substituents.

The reactions in nitrobenzene alcohol (80 : 20 V/V) are uniformly faster than in nitrobenzene. The higher reactivity in the mixed solvent is due to the protic character of the solvent.

TABLE I

Second order rate constants ( $l. \text{ moles}^{-1} \text{ min}^{-1}$ ) for the reaction of tertiary amines with benzyl halides  
 Solvent : Nitrobenzene Temperature  $80^\circ \text{C}$ .

| Base                  | Pka* | Benzyl chloride    | p-Methyl benzyl chloride | p-Chloro-benzyl chloride | o-Chloro-benzyl chloride |
|-----------------------|------|--------------------|--------------------------|--------------------------|--------------------------|
| Pyridine              | 5.17 | 0.006<br>(.014)    | 0.010<br>(.030)          | 0.005<br>(.011)          | 0.005<br>(.011)          |
| $\alpha$ -Picoline    | 5.97 | 0.001<br>(.0025)   | 0.0021<br>..             | 0.00075<br>(.0025)       | 0.00083<br>(.0025)       |
| $\beta$ -Picoline     | 5.68 | 0.0112<br>(.0235)  | 0.0132                   | 0.0085<br>(.018)         | 0.011<br>(.0225)         |
| $\gamma$ -Picoline    | 6.02 | 0.0121<br>(.040)   | ..                       | 0.0090<br>(.020)         | 0.0121<br>(.023)         |
| Quinoline             | 4.85 | 0.00049<br>(.0016) | 0.0018<br>(.0025)        | 0.00049<br>(0.00083)     | 0.00040<br>(.0009)       |
| Isoquinoline          | 5.14 | 0.0083<br>(.016)   | 0.011<br>(.031)          | 0.0073<br>(.014)         | 0.0079<br>(.016)         |
| 2-Methyl quinoline    | 5.42 | 0.0016             | 0.0029                   | 0.0011                   | 0.0017                   |
| 3-Methyl isoquinoline | 5.64 | 0.0011             | 0.0025                   | 0.00067                  | 0.00085                  |

\* At  $25^\circ \text{C}$  in aqueous medium taken from *Physical Methods in Organic Chemistry*, Vol. I, by Braude and Nachod for purposes of comparison.

Values in parentheses are rate constants in Nitrobenzene-alcohol (80:20 V/V) mixture at  $80^\circ \text{C}$ .

*Application of Tommila equation.*—Application of the Tommila equation<sup>6</sup> suggests a positive reaction centre in benzyl chloride as seen from the nature of log  $K_s/k_u$  values given in Table II.

TABLE II

Ratio of rate constants of substituted and unsubstituted benzyl chlorides in nitrobenzene and nitrobenzene-alcohol

| Substrate   | X                 | Base      | log $K_s/k_u$ |                                   |
|---|-------------------|-----------|---------------|-----------------------------------|
|   |                   |           | Nitro-benzene | Nitro-benzene ethanol (80:20 V/V) |
| X-C <sub>6</sub> H <sub>4</sub> -CH <sub>2</sub> Cl | P-CH <sub>3</sub> | Pyridine  | -0.22         | -0.32                             |
|   | P-Cl              |           | -0.08         | -0.10                             |
|   | P-CH <sub>3</sub> | Quinoline | -0.57         | -0.20                             |
|   | P-Cl              |           | ..            | -0.28                             |
|   | O-Cl              |           | -0.10         | -0.25                             |

TABLE III

Nucleophilic reactivity constants of tertiary bases

$\alpha$  (For Benzyl chloride) = 3.53,

$\beta$  (for Benzyl chloride) = -0.128

Substrate : Benzyl chloride Temperature :  $80^\circ \text{C}$   
 Solvent : Nitrobenzene

| Base               | H    | Nucleophilicity | $E_n$          |
|--------------------|------|-----------------|----------------|
| Pyridine           | 6.91 | 4.80<br>(4.80)  | 1.44<br>(1.44) |
| $\alpha$ -Picoline | 7.71 | 3.94<br>(3.90)  | 1.25<br>(1.24) |
| $\beta$ -Picoline  | 7.42 | 5.04<br>(5.11)  | 1.51<br>(1.53) |
| $\gamma$ -Picoline | 7.76 | 5.33<br>(5.16)  | 1.59<br>(1.55) |
| Quinoline          | 6.59 | 3.72<br>(3.55)  | 1.16<br>(1.11) |
| Isoquinoline       | 6.88 | 4.88<br>(4.96)  | 1.45<br>(1.47) |
| 2-Methyl quinoline | 7.16 | 3.96            | 1.24           |
| 3-Methyl quinoline | 7.38 | 4.02            | 1.26           |

Values in parentheses are in nitrobenzene-alcohol (80:20 V/V).

*Application of linear free energy relationship.*—Swain-Scott<sup>7</sup> and Edwards<sup>8</sup> equations have been applied to the kinetic data and parameters like nucleophilicity and  $E_a$  values for the bases used have been computed in nitrobenzene and nitrobenzene-alcohol mixtures which help in understanding the mechanism of the process to some extent<sup>9</sup>. The parameters are given in Table III.

The parameters are identical in nitrobenzene and nitrobenzene-alcohol mixture demonstrating the operation of identical mechanism in both the solvent systems.

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# PHYSICO-CHEMICAL STUDIES OF SOME BIVALENT METAL ION CHELATES OF N-ACETYLACETONE ORTHANILIC ACID

THE results of physico-chemical studies on the chelates of N-Acetylacetone orthanilic acid ( $H_2AO$ ) with some bivalent metal ions are reported in this note.

Orthanilic acid supplied by SCHUCHAROT MUNCHEN was used. All other compounds were of BDH grade. The molecular weights, elemental analysis, magnetic susceptibility and electronic absorption spectra were determined by standard methods as reported earlier<sup>1</sup>.

*Preparation of  $H_2AO$  and its metal chelates.*— $H_2AO$  Schiff base was prepared by the method of Pfeiffer *et al.*<sup>2</sup>. The metal chelates were synthesised by the method of Yamada *et al.*<sup>3,4</sup> and their pyridine solvates by the method reported earlier<sup>1</sup>.

Analysis: Found C, 51.81; H, 4.99; N, 5.47; S, 12.41. Calcd. for  $C_{11}H_{13}NSO_4$ , C, 51.96; H, 5.11; N, 5.51; S, 12.59%, m.p. 160°.

*Results and Discussions.*—Elemental analysis, molecular weight and magnetic data of the hydrated chelates are given in Table I.

The molecular weight data indicate that Fe (II), Co (II), Ni (II) and Cu (II) chelates possess the composition  $[MLX_3]$  where M represents the metal ion,  $X = H_2O$  or Py and  $LH_2 = [C_{11}H_{13}NSO_4]$ . The magnetic moment values reveal the presence of 4, 3, 2 and 1 unpaired electrons in the Fe (II), Co (II), Ni (II) and Cu (II) chelates respectively. The high magnetic moment of Co (II) chelate appears to be due to spin-orbit coupling.

The electronic absorption spectra of these chelates were studied both in dioxane and pyridine solu-

TABLE I

Elemental analyses, molecular weights and magnetic moments of the metal chelates of N-acetylacetone-orthanilic acid

| Metal chelate                               | Mol. wt.    | Metal %       | N %         | H <sub>2</sub> O % | $\mu_{eff}$ (B.M.)<br>at 298° K |
|---|-------------|---------------|-------------|--------------------|---------------------------------|
| [Fe( $C_{11}H_{13}NSO_4$ ) $X_3$ ]          | 357 (362)   | 14.32 (15.46) | 3.81 (3.86) | 14.87 (14.91)      | 5.48                            |
| [Co( $C_{11}H_{13}NSO_4$ ) $X_3$ ]          | 369 (365)   | 16.01 (16.16) | 3.80 (3.83) | 14.71 (14.79)      | 5.12                            |
| [Ni( $C_{11}H_{13}NSO_4$ ) $X_3$ ]          | 365 (364.7) | 16.80 (16.92) | 4.07 (4.11) | 14.76 (14.80)      | 3.11                            |
| [Cu( $C_{11}H_{13}NSO_4$ ) $X_3$ ]          | 360 (369.5) | 17.00 (17.18) | 3.69 (3.78) | 14.51 (14.61)      | 1.89                            |
| [Pd( $C_{11}H_{13}NSO_4$ ) X]               | 370 (376)   | 28.07 (28.19) | 3.61 (3.72) | 4.71 (4.79)        | ..                              |
| [Zn( $C_{11}H_{13}NSO_4$ ) X]               | 339 (335.4) | 19.31 (19.49) | 4.10 (4.17) | 5.30 (5.36)        | ..                              |
| [Cd( $C_{11}H_{13}NSO_4$ ) X]               | 377 (382.4) | 29.27 (29.39) | 3.59 (3.66) | 4.59 (4.70)        | ..                              |
| [UO <sub>2</sub> ( $C_{11}H_{13}NSO_4$ ) X] | 545 (540)   | 49.90 (50.00) | 2.54 (2.59) | 3.31 (3.33)        | ..                              |

Calculated values are given in parentheses. X refers to H<sub>2</sub>O. Zn (II), Pd (II), Cd (II), UO<sub>2</sub> (II) chelates were found to be diamagnetic, as expected.

ions. The frequencies and the corresponding transitions observed are summarized below.

| Complex | Observed $\nu_{\text{max}}$ (cm <sup>-1</sup> )<br>Dioxane-Pyridine | Possible transitions   |
|---------|---|--|
| Fe(II)  | 1169, 1160  | $T_{2g} \rightarrow E_g$   |
| Co(II)  | 1590, 1170<br>1170, 1160  | $T_{2g}(F) \rightarrow T_{2g}(F)$<br>$T_{2g}(F) \rightarrow A_{1g}(F)$                 |
| Ni(II)  | 1470, 1370<br>1150, 1140  | $T_{2g} \rightarrow T_{2g}(F)$<br>$A_{1g} \rightarrow T_{2g}(P)$                       |
| Cu(II)  | 1470, 1350  | $E \rightarrow T_{2g}$   |
| Pd(II)  | 1110, 1100<br>1070, 1100<br>1150, 1120                              | $A_{1g} \rightarrow B_{1g}$<br>$A_{1g} \rightarrow E_g$<br>$A_{1g} \rightarrow A_{2g}$ |

An I.R. study of  $H_2AO$  has shown three bands at 1169 cm<sup>-1</sup>, 1170 cm<sup>-1</sup> and 1150 cm<sup>-1</sup> which can be assigned to the presence of sulphonic acid ( $SO_3H$ ),  $C=N$  and enolic  $-OH$  respectively. In all the metal chelates under investigation the bands at 1169 cm<sup>-1</sup> and 1150 cm<sup>-1</sup> could not be located which suggests their elimination due to complexation.

The presence of chelated water molecules in these compounds has been confirmed by their I.R. spectra in the region 3.1-4.0  $\mu$ .

These data supported by their magnetic moments confirm an octahedral structure for  $Fe(II)$ ,  $Co(II)$ ,  $Ni(II)$  and  $Cu(II)$  chelates.

The  $Zn(II)$ ,  $Pd(II)$ ,  $Cd(II)$  and  $UO_2(II)$  also form 1 : 1 chelates and their composition can be expressed by the formula  $[MLN]$ , where M denotes the metal ion,  $LH_2 = [C_5H_5NSO_3]$  and  $N = H_2O$  or  $Py$ . The hydrated chelates and their pyridine solvates have been found to be diamagnetic. These results can be explained by assigning a tetrahedral structure for  $Zn(II)$  and  $Cd(II)$  chelates and an octahedral structure for  $UO_2(II)$  chelate. The diamagnetic behaviour of the  $Pd(II)$  chelate indicates a square-planar stereochemistry around the central  $Pd(II)$  ion. These results are also in agreement with an earlier finding<sup>1</sup>.

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# SR ISOTOPE RATIOS OF THE ALBERT BASALT-IMPLICATIONS IN THE PETROGENESIS OF THE FOCAL PEAK SHIELD VOLCANO, SOUTHEAST QUEENSLAND, AUSTRALIA

IN southeast Queensland and northeast New South Wales, Australia, an extinct shield volcano, named the Focal Peak Shield Volcano, exists<sup>1</sup>. The volcanics and associated intrusives of this shield volcano are late Oligocene in age. Extrusives include, on its eastern side, the alkaline Albert Basalt and the metaluminous Mt. Gillies Rhyolite. The petrogenetic relationship between these two formations will be examined here in the light of available Sr isotope ratios. Most of the extrusives of the Albert Basalt are hawaiites, using the classification system proposed by Coombs and Wilkinson<sup>2</sup> assuming a 1-50%  $Fe_2O_3$  content<sup>3</sup>. A few basanites, alkali olivine basalts, mugearites and even a brecciated trachyte exist.

Only limited Sr isotopic information is available for the Albert Basalt (Table I).  $Sr^{87/86}$  ratios for the formation are comparable to those of continental alkali basalts and slightly higher than those normally expected for oceanic alkali basalts<sup>4</sup>. These data suggest that either the Albert Basalt was :

- (1) derived from mantle material with a high  $Sr^{87/86}$  ratio, or
- (2) contaminated by the addition of silic material, or
- (3) contaminated by selective diffusion of certain elements, including Sr, from continental crust into the basaltic magmas, or
- (4) selective migration of radiogenic Sr from crustal rocks into the magmas.

Major and trace element geochemistry discounts the possibility of contamination of the basaltic magmas by the addition of silic material alone being the major causative factor. It appears that factors (3) and (4) above are the most likely causes. Isotopic contamination would probably result not only during the active transportation of the basaltic magmas to the earth's surface, but also, to a very important degree, during temporary halts of transportation within both the mantle (if isotopically heterogeneous) and, more importantly, within the continental crust. It is considered, from field and detailed geochemical studies, that the Mt. Gillies Rhyolite was derived from the Albert Basalt which includes both undersaturated and saturated representatives. Sufficient time is required to pass for the processes of strong fractionation to generate rhyolitic differentiates from basaltic parents. Consequently, the concept of halts in the transportation of magmas is important in petrogenetic discussions. In addition, it should

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be noted that the plagioclase-clinopyroxene-olivine thermal barrier, often separating the fields of saturated and undersaturated basalts, appears not to have prevented siliceous differentiates being derived from the more undersaturated representatives of the Albert Basalt. This is probably due to some of the formation's basaltic magmas passing from the undersaturated to saturated field at  $P \geq 20 \text{ kb.}^2$  (i.e., at a depth approximately  $\leq 65 \text{ km}$ ) where the thermal barrier is inoperative.

In summary then Sr isotopic evidence has been useful in studies of the petrogenetic history of the Focal Peak Shield Volcano in which the Mt. Gillies Rhyolite is believed to be a strongly fractionated differentiate of the alkaline Albert Basalt. Halts in transportation of the differentiating magmas towards the earth's surface is believed to be of great importance in the petrogenesis of the rhyolitic formation.

TABLE I

$\text{Sr}_{87/86}$  ratios for the Albert Basalt\*

| Sample No.** | Rock Type             | $\text{Sr}_{87/86}$ |
|--------------|-----------------------|---------------------|
| 33,063       | Hawaiite              | 0.7067              |
| 33,065       | Mugearite             | 0.7042              |
| 33,066       | Alkali olivine basalt | 0.7045              |

\*  $\text{Sr}_{87/86}$  has been calculated on the basis of a late Oligocene age<sup>1</sup>.

\*\* Sample numbers are for the University of Queensland, Department of Geology and Mineralogy Rock Catalogue.

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# ON THE SIGNIFICANCE OF ENHANCED GLUTAMINE SYNTHETASE AND ITS REGULATION DURING AESTIVATION IN *PILA GLOBOSA*

In aestivating *Pila globosa* the protein content decreases and the general amino acid pool increases of which certain amino acids alone predominate<sup>1</sup>. Uric acid level increases<sup>2,3</sup> while urea content decreases<sup>4</sup> in all the tissues during aestivation indicating that the protein nitrogen is probably metabolized to uric acid during aestivation. However there are no reports on the activity levels of the enzymes concerned with the synthesis of uric acid during aestivation. The present report concerns the distribution and the activity of glutamine synthetase during aestivation, since this enzyme level determines the glutamine level which is known to contribute to N-3 and N-9 of the uric acid molecule.

Glutamine synthetase activity in the hepatopancreas, mantle and foot of the active and three months aestivated *Pila globosa* was estimated by the method of Iqbal and Wu<sup>4</sup>.

It was observed that the synthesis of glutamine from glutamic acid is limited to hepatopancreas since the other two tissues, namely, mantle and foot had no detectable activity of the enzyme. Thus in mantle and foot the rate of glutamine synthesis is slowest thereby limiting uric acid biosynthesis. Such control mechanisms limiting the entire pathway by some slow steps in reaction sequences are not uncommon in animal biochemistry. The low level of this enzyme will be responsible for lower glutamate metabolism into glutamine thus resulting in accumulation of glutamate<sup>1,5</sup> reported during aestivating *Pila globosa*.

There is a 100% increase in the glutamine synthetase in hepatopancreas of the 30 days aestivating *Pila globosa* and the activity is maintained at that level even in 3 months aestivating snails. In active snails the ammonia produced in the metabolism is constantly excreted thereby averting the possible ammonia toxicity to the tissue metabolism. In the absence of such ammonia toxicity the  $\alpha$ -ketoglutarate could have been metabolized without the necessity of being converted to glutamate. In the present investigation the aestivating snail, since the possibility of excretion is less, there is every danger of accumulation of ammonia to the level of lethality. To prevent this catastrophe the aestivating metabolism is geared to alternate stepping up of processes that knock out ammonia which could be as follows.

$\alpha$ -Ketoglutarate dehydrogenase complex was shown to be inhibited by  $\text{NADH}_2$ <sup>13</sup>. The increased cytoplasmic and mitochondrial  $\text{NADH}_2/\text{NAD}$  ratio have been reported in the liver of turtle and rat



TABLE I

Glutamine synthetase activity in tissues of active and different periods of aestivated *Pila globosa*

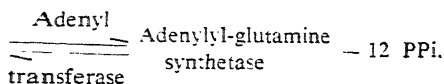
| Sl. No. | Tissues        | Active     | 1 Month aestivated                    | 2 Months aestivated                 | 3 Months aestivated                    |
|---------|----------------|------------|---------------------------------------|-------------------------------------|--|
| 1.      | Foot           | —          | —                                     | —                                   | —                                      |
| 2.      | Mantle         | —          | —                                     | —                                   | —                                      |
| 3.      | Hepatopancreas | 6.3 ± 1.78 | 12.6 ± 1.1<br>—100.00%<br>$p > 0.001$ | 12.1 ± 1.1<br>—92.6%<br>$p > 0.001$ | 11.33 ± 2.23<br>—79.86%<br>$p > 0.001$ |

## Note:

1. — : indicates activity below the level of detection.
2. — : indicates standard deviation (S.D.).
3. —% : indicates percent over active.
4.  $p$ — : level of significance.

during hypoxia<sup>14,15</sup>. Moreover, succinate dehydrogenase activity was reported to decrease in aestivation<sup>11,12</sup> indicating that the formation of succinate through  $\alpha$ -ketoglutarate dehydrogenase complex is less. Under these conditions it is likely that the amination of the  $\alpha$ -ketoglutarate by glutamate dehydrogenase is preferred to oxidation by  $\alpha$ -ketoglutarate dehydrogenase. Increased glutamate dehydrogenase reported during aestivation<sup>16</sup> is consistent with the above inference. Glutamic acid is shown to be a potent inhibitor of the nervous system<sup>17</sup> and many enzyme systems in *Pila globosa*. Hence it is converted to less toxic glutamine.

The activity pattern of glutamine synthetase is modulated by adenylation of the enzyme by ATP. This process is catalyzed by adenylyl transferase. The increase in the level of glutamine and ATP activate this enzyme which results in the elevated levels of glutamine synthetase-AMP complexes<sup>7</sup>. The adenylation complex has low activity and consequently the formation of further glutamine is reduced<sup>8,9</sup>. Glutamine synthetase — 12 ATP



In the aestivated animal tissues ATP and glutamine levels were high<sup>10</sup> which under normal conditions activate adenylyl transferase, resulting in a decrease of glutamine synthetase activity. On the contrary, an increase in the activity of glutamine synthetase is observed in aestivated animal tissues indicating nonadenylation of the enzyme possibly by the stopped up deadenylation.  $\alpha$ -Ketoglutarate and ammonia are known to activate deadenylylating systems<sup>6</sup>. This peculiar shift is probably for meeting the emergency survival demand against ammonia toxicity.

Thus in the aestivating *Pila globosa* though the conditions are favourable for adenylation of

glutamine synthetase as a consequence of high levels of ATP and glutamine adenylyl transferase is not active. Hence there is a shift in the pattern of enzyme regulation towards the activation of deadenylylating enzyme or insensitization of adenylylating system.

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# CHEMICALLY INDUCED SPERM GRANULOMA IN RAT

RECENTLY Cooper and Jackson<sup>1</sup> reported that ethylenedimethane sulphonate (EDS), an alkylating agent, caused sperm retention cysts in the rat epididymis.  $\alpha$ -chlorohydrin an active alkylating chemical possessing the specific biological property of "functionally" inactivating epididymal sperm frequently induces pathological changes in the epididymis.

The present investigation is concerned to study the pathological changes involved in the  $\alpha$ -chlorohydrin induced sperm retention cysts/or granuloma in rat and to discuss the biologic significance of the phenomena observed.

$\alpha$ -chlorohydrin (3-chloro-1, 2-propanediol) (sp. gr. 1.326) was supplied by the Upjohn Company, Kalamazoo, Michigan, in 0.25% aqueous (1.3 g/ml) methyl cellulose. A working solution was made by diluting the stock solution with distilled water. Ten adult male Wistar rats from the randomly mated colony were given  $\alpha$ -chlorohydrin orally (25 mg/kg/day for 24 days). The controls received distilled water only. The animals were given rat food (Purina chow: Hindustan Lever Private Ltd.), wet gram and water *ad libitum*.

Twenty-four hours after the administration of the final dose of  $\alpha$ -chlorohydrin, the rats were killed by rapid decapitation. Final body weight, and the weights of testis, epididymis, seminal vesicle, ventral prostate and levator ani muscles were recorded. The epididymis were examined with naked eye for cysts formations.

Grossly sperm retention cysts were evident as yellow nodular masses varying in size from 1.25 mm to 3.5 mm in the greatest dimension. The most frequent site was the lower pole of the epididymis.

In histologic preparations, the cyst consisted of a central pool of sperm surrounded by macrophages and histocytes (Fig. 1). The epididymis contained

mononuclear cells, spermatocytes and multinucleated spermatid elements. Distension of the coils of ductus-epididymis and atrophy of the lining-epithelium were conspicuous. Fibrosis and hyalinization of the epididymal tubules were common.

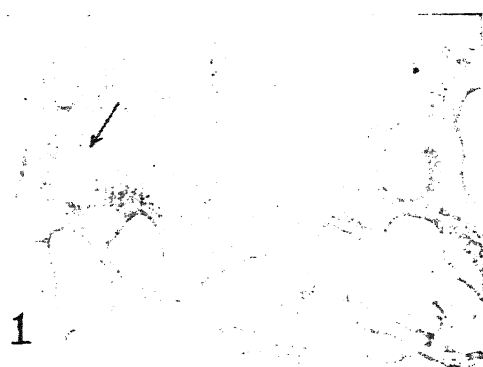


FIG. 1. Showing the sperm retention cyst in the cauda epididymis of rat after  $\alpha$ -chlorohydrin treatment (Total dose 24 mg). Note the central pool of sperms surrounded by macrophages (—)  $\times 24$  HE.

The present investigation points out the possible cause for chemically induced spermatid granuloma formation is, the damage to the epididymal epithelium for the leakage of sperm. The sperm may then become involved with the inflammatory process in the stroma, resulting in the formation of the sperm granuloma. From a practical point of view, spermatid granulomas are important because they stimulate other lesions, particularly tuberculous epididymitis<sup>2</sup>.

In the rat, the epididymis is androgen dependent.  $\alpha$ -chlorohydrin produces marked inhibition of the accessory sexual structures (seminal vesicle, ventral prostate and levator ani muscles; Table I) indicating pharmacological action on the androgenic

TABLE I

Changes in body weight, the weights of testes and accessory sex-organs of adult male rats after oral treatment with  $\alpha$ -chlorohydrin\*

| Group | Treatment   | Initial body wt. g | Final body wt. g | Testes wt. (mg) | Seminal vesicle wt. (mg) | Ventral prostate wt. (mg) | Epididymis wt. (mg) | Levator ani muscle wt. (mg) |
|-------|---|--------------------|------------------|-----------------|--------------------------|---------------------------|---------------------|-----------------------------|
| 1.    | Control (10)  | 245 $\pm$ 19       | 252 $\pm$ 11     | 2297 $\pm$ 200  | 735 $\pm$ 95             | 286 $\pm$ 20              | 788 $\pm$ 30        | 128 $\pm$ 13                |
| 2.    | $\alpha$ -Chlorohydrin (Total dose 120 mg 24 days) (10) | 237 $\pm$ 18       | 215 $\pm$ 9      | 981 $\pm$ 78†   | 309 $\pm$ 47†            | 80 $\pm$ 13†              | 462 $\pm$ 14†       | 64 $\pm$ 9†                 |

\* 5 mg/day for 24 days.

†  $P < 0.01$  compared with controls.

Figures in parentheses represent the number of animals examined. All figures  $\pm$  S.E.M.

function of the leydig cells. Chronic administration of  $\alpha$ -chlorohydrin produce spermatocoele<sup>2</sup>, which may be important in relation to antifertility of this compound.

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#### A NOTE ON THE COMPARATIVE STUDY OF FREE AMINO-ACIDS CONTENT BETWEEN WILD SALT TOLERANT RICE AND CULTIVATED RICE VARIETIES

Wild rice (*Oryza coarctata*) grows profusely on saline marshy area (electrical conductivity above 25 m.mhos/cm) near the institute farm. An attempt has been made to study the physiology of salt tolerance of this wild rice. This note reports free amino-acid content of wild and cultivated rice varieties.

For a comparative study along with the wild rice, a local salt-tolerant rice variety Damodar, and a high yielding rice variety Jaya was selected. Plant samples were collected from young seedlings (33 days old). Free amino-acids were analysed from fresh samples by paper chromatographic method as suggested by Plaisted<sup>1</sup>. Leaf and stem were analysed separately. Free amino-acid content of the rice varieties are given in Table I.

The results show that alanine, serine and glycine, histidine and arginine, and proline content of wild salt-tolerant rice—*Oryza coarctata*—is more as compared to cultivated rice varieties. It is interesting to note that proline content of *Oryza coarctata* is quite high compared to the other varieties. From comparative rates of proline accumulation in various plant organs Singh *et al.* (1973) postulated that a water deficit or osmotic stress induces proline accumulation in the leaves from where it is translocated to the roots and other plant organs. Stewart *et al.* (1966) suggested that proline may be the major source of energy and nitrogen during immediate post-stress metabolism. From this it can be concluded that possibly proline which accumulates under osmotic stress condition is

TABLE I

*A comparative study of free amino-acids content between wild salt-tolerant rice and cultivated rice varieties (in microgram gram of dry matter)*

| Name of the Amino-acids      | <i>Oryza coarctata</i> (wild rice) |          | Damodar  |          | Jaya     |        |
|------------------------------|------------------------------------|----------|----------|----------|----------|--------|
|                              | Stem                               | Leaf     | Stem     | Leaf     | Stem     | Leaf   |
| Alanine                      | 1,754.20                           | 52.17    | 983.00   | 1,855.52 | 1,426.55 | 142.47 |
| $\beta$ -Alanine             | 156.60                             | 90.33    | 255.30   | 109.52   | 713.24   | ..     |
| $\gamma$ -Amino butyric acid | 350.80                             | 99.51    | 105.00   | 130.00   | 271.32   | ..     |
| Aspartic acid                | 701.40                             | 105.79   | 367.62   | 288.92   | 545.43   | 36.93  |
| Asparagine                   | 467.60                             | 48.30    | 192.33   | 53.27    | 265.73   | ..     |
| Glutamic acid                | 327.40                             | 115.45   | 784.60   | 775.63   | 727.24   | 50.62  |
| Histidine and arginine       | 1,204.40                           | 40.09    | 149.23   | 60.00    | 153.88   | ..     |
| Leucine                      | 134.40                             | 140.09   | 190.70   | 139.12   | 167.83   | 86.65  |
| Lysine                       | 140.20                             | 55.55    | ..       | 35.72    | ..       | ..     |
| Methionine and valine        | 327.40                             | 64.73    | 184.62   | 139.10   | 135.60   | 67.83  |
| Phenylalanine                | 32.60                              | 24.63    | 72.30    | 32.65    | 173.42   | ..     |
| Proline                      | 1,590.60                           | 1,468.59 | 384.62   | 76.00    | 307.65   | ..     |
| Serine and glycine           | 1,263.00                           | 169.08   | 969.24   | 501.98   | 951.00   | 83.28  |
| Threonine                    | 249.00                             | 62.80    | 184.66   | 94.23    | 116.00   | ..     |
| TOTAL                        | 8,844.60                           | 2,537.31 | 4,823.22 | 4,291.66 | 5,954.89 | 467.78 |

supplying energy for growth and survival under saline condition and thereby inducing salinity resistant to crops.

The author is thankful to Mr. P. K. Basu Roychoudhury of Calcutta University, Department of Agriculture, for his help in this investigation.

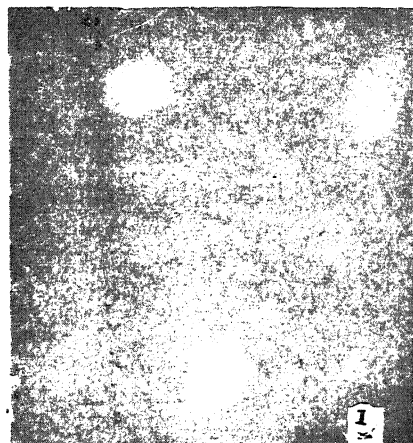
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#### PRENATAL DETECTION OF FETAL SEX\*

In 1956 Fuchs and Riis showed the possibility of foetal sex determination from amniotic fluid cells using Barr body technique. In 1971 Khudr and Benirschke used fluorescence studies to locate Y-chromosome (Y-body) in the nuclei of amniotic fluid cells in male cases.



Figs. 1-2

The amniotic fluid cells are of fetal origin and derived mainly from fetal skin and amnion. The present paper deals with the use of simple and rapid techniques (Barr body and fluorescence method) available for prenatal sex determination.

**Material and Methods.**—The amniotic fluid samples were obtained from 15 patients undergoing therapeutic abortion by intrauterine prostaglandin or by saline injection between 10 to 18 weeks of gestation. One sample was obtained from a patient who had two sons and 8 other male members in

the family suffering from leukodystrophy. All abortions were done for psychiatric and health reasons.

The volume of amniotic fluid sample obtained varied from 5 ml to 12 ml depending on the month of pregnancy, and the technique used for abortion.

The sample was centrifuged at 1000 r.p.m. for 10 minutes. After discarding the supernatant, the cell pellet was resuspended and fixed in acetic acid alcohol (3 parts methanol — 1 acetic acid) for 20 minutes. The smears were prepared and some of the slides were stained in Giemsa stain for X-chromatin and the rest was stained in 0.5% quinacrine dihydrochloride (Atebrin) for fluorescence microscopy to study Y-body.

**Results and Comments.**—Results were confirmed by the gross examination of fetuses, mainly of external genitalia. The results agreed in all the cases except one, where the sample was not sufficient for the satisfactory preparation.

The amniotic fluid cells of male fetuses showed a single fluorescent body usually located peripherally sometimes eccentrically within the nucleus (Fig. 1). In case of a female, amniotic fluid cells showed typical Barr body (Fig. 2) but no such fluorescent

body. One hundred cells were scanned from each preparation and in the nuclei of male fetuses a typical fluorescent body was found in 20% to 30% of the cells and in the nuclei of female fetuses 20% to 60% of typical Barr body was found. The variation in number may be due to presence of different types of cells. In a case of leukodystrophy the amniotic fluid cells showed male fetus and the patient decided to continue pregnancy. She delivered a male child which confirmed our prediction of the sex.

This preliminary study shows that the method used may be of great help in determining the sex of the fetus at the early stage of pregnancy. It is a test which can be performed in less than one hour.

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### RELATION OF SPORULATION TO FUSARIC ACID PRODUCTION IN MUSKMELON WILT PATHOGEN

FUSARIC acid (FA), a wilt toxin, is produced by many fusaria both *in vitro* and *in vivo*. From this laboratory Bhaskaran and Prasad<sup>1</sup> reported the *in vitro* production of FA by the muskmelon wilt pathogen [*Fusarium oxysporum* f. *melonis* (Leach and Currence) Snyder and Hans.]. Interestingly the toxin production was more in media which favoured good sporulation<sup>2</sup>. Studies on the relation between sporulation and fusaric acid production were made and the results are reported here.

Hundred ml aliquot of sterilized Czapek's medium in 250 ml conical flasks were inoculated with 8 mm disc of the actively growing fungus and incubated at room temperature ( $28 \pm 2^\circ \text{C}$ ). At the end of incubation period, the flasks were shaken in a rotary shaker for 30 min and the spore load was estimated by Haemoagglometer counts. The content was passed through a double layered cheese cloth, washed with distilled water. The mycelial mat was separated from cheese cloth, blotted and weighed. The filtrate was centrifuged to separate spores from culture filtrate. The fusaric acid in culture filtrate, mycelium and spores was detected following the respective methods<sup>3,4</sup>. The presence of FA was confirmed by paper chromatography. The influence of certain media, viz., Czapek's, Coon's, Horne and Mittar's and Park's suggested for *Fusarium*<sup>5</sup> was tried for assessing growth, sporulation and FA level.

The effect of incubation periods on growth, sporulation and FA production is shown in Table I and the effect of different media on growth and FA production is presented in Table II. Among the media tested, Czapek's medium favoured relatively higher sporulation and FA production. Growth

TABLE I

Effect of incubation period on growth, sporulation and fusaric acid (FA)\* production by *Fusarium oxysporum* f. *melonis*, when grown in Czapek's medium

| Incubation period in days | Mycelial dry weight mg/100 ml | FA in mycelium | Number of spores/ml | FA in spores | FA in culture filtrate |
|---------------------------|-------------------------------|----------------|---------------------|--------------|------------------------|
| 10                        | 175                           | 274.5          | 15,000              | 310.0        | 610.0                  |
| 15                        | 227                           | 334.0          | 38,000              | 615.0        | 928.3                  |
| 20                        | 232                           | 330.0          | 53,000              | 980.0        | 1240.0                 |
| 25                        | 240                           | 332.0          | 52,000              | 950.0        | 1220.0                 |
| 30                        | 245                           | 335.0          | 49,000              | 890.5        | 1195.0                 |

\* FA in inhibition annules (mm<sup>2</sup>).

TABLE II

Effect of different media on growth, sporulation and FA\* production by *Fusarium oxysporum* f. *melonis*

| Media used                | Mycelial dry weight (mg/100 ml) | FA in mycelium | Number of spores/ml | FA in spores | FA in culture filtrate |
|---------------------------|---------------------------------|----------------|---------------------|--------------|------------------------|
| Coon's medium             | 247                             | 285.0          | 42,000              | 612.0        | 1130.2                 |
| Czapek's medium           | 238                             | 280.0          | 51,000              | 845.0        | 1230.8                 |
| Horne and Mittar's medium | 312                             | 315.0          | 28,000              | 394.5        | 829.5                  |
| Park's medium             | 215                             | 225.0          | 32,000              | 480.0        | 890.5                  |

\* FA in inhibition annules (mm<sup>2</sup>).

studies in Czapek's broth indicated maximum sporulation and FA production on 20th day and growth on 30th day. Thus a parallelism existed between sporulation and FA production by this pathogen. Sandhu<sup>6</sup> demonstrated that FA is a product of active metabolism, probably ultimately connected with tricarboxylic acid cycle. The accelerated metabolism during sporulation may be the cause of more FA production. The toxin level in mycelium and conidia showed a marked variation. FA in mycelium and conidia was first reported<sup>2</sup> in *Fusarium oxysporum* f. *vasinfectum*. Toxin in mycelium and

conidia was estimated for the first time in this pathogen. Toxin content of conidia was more than that of the mycelium produced per unit quantity of medium. These results further substantiate that there is a definite positive correlation between sporulation and toxin production.

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#### A BIOLOGICAL APPROACH TO THE CONTROL OF MAIZE BORER *CHILO ZONELLUS* (SWINHOE)

ISOLATIONS made from the body surface of the borer, *Chilo zonellus* (Swinhoe), its frass deposits and rotted maize stalk in the vicinity of borer tunnels gave the following fungi: *Cephalosporium acremonium*, *Fusarium moniliforme*, *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., *Trichoderma* sp., *Botrytis* sp. and a *Fusarium* sp.. Among the bacterial forms were *Pseudomonas* sp., and *Bacillus* sp.

A species of *Fusarium* which was identified as *Fusarium aleyrodis* Petch was found to be pathogenic on this insect, others were not.

For confirmation of the pathogenicity of *F. aleyrodis*, originally isolated from the insect body surface, field collected larvae ranging from 2nd to 4th instar were individually immersed in fungal spore suspension. A control was maintained. The larvae were fed with finely chopped pieces of maize stem. Whitish fungal mat appeared gradually on the insect's body. Single spore isolation from this hyphal mat yielded *F. aleyrodis* Petch. Repeated patho-

genicity tests also confirmed it. The larvae during fungal colonization became sluggish, stopped feeding, turned brown and succumbed within 2-5 days of infection. Toxicity of the fungus is believed to be responsible for pathogenic action on the insect<sup>1,2,3</sup>. Mechanism of fungal action could be ascertained by observing separately the effect of fungal spore spray and its crude toxin on the insect larvae.

Spore spraying showed that by the 7th day the insects were killed (Table I). Fungal mat appeared on the 8th day and on the 9th day the entire insect body was covered with sporulating hyphae.

TABLE I

Effect of fungal spore spray on larvae of *Chilo zonellus* (20 larvae were used in each replication)

| Days | Number of larvae dead                                  |     |     |     |
|------|--|-----|-----|-----|
|      | Replications   |     |     |     |
|      | 1  | 2   | 3   | 4   |
| 1    | ..   | ..  | ..  | ..  |
| 2    | ..   | ..  | ..  | ..  |
| 3    | ..   | ..  | ..  | ..  |
| 4    | ..   | ..  | ..  | 1   |
| 5    | 5  | 7   | 6   | 6   |
| 6    | 8  | 8   | 9   | 7   |
| 7    | All  | All | All | All |
| 8    | Fungal mat seen on the insect                          |     |     |     |
| 9    | Fungal mat as well as spores found on all the insects. |     |     |     |

Crude toxin obtained from *F. aleyrodis* culture grown in Richard's liquid medium, when applied topically on the larvae, killed them. The toxin-treated insects stopped taking food, showed tetanic reaction and subsequently died. The larvae started dying right from the very beginning reaching the maximum on the 13th day (Table II). Mortality rate progressed with number of days of incubation.

*Chilo zonellus* has been found to be attacked and pathogenically colonized by another fungus *Beauveria densa* Link.<sup>4,5</sup>. *Chilo partellus* was effected by *Aspergillus flavus* and a *Fusarium* sp.<sup>1</sup>. The pathogenic attack of *Chilo zonellus* by *Fusarium aleyrodis* Petch, adds to the list of fungi parasitic on insects. Spraying of the insect with spore suspension or treatment with crude toxin of this fungus could be of biological control value.

TABLE II  
Effect of fungal toxin on *Chilo zonellus*

| Period of exposure to toxin (Days) | Average number of larvae dead when sprayed with toxin (20 larvae) |    |    |    |    |    |    |
|------------------------------------|---|----|----|----|----|----|----|
|                                    | Days of incubation  |    |    |    |    |    |    |
|                                    | 0   | 3  | 5  | 7  | 9  | 11 | 13 |
| 1                                  | ..  | .. | .. | .. | .. | .. | 1  |
| 2                                  | 1   | .. | 2  | 3  | 3  | 5  | 7  |
| 3                                  | 1   | 4  | 4  | 11 | 13 | 13 | 14 |
| 4                                  | 1   | 4  | 9  | 15 | 15 | 15 | 18 |
| 5                                  | 5   | 4  | 10 | 16 | 16 | 19 | 20 |
| 6                                  | 5   | 4  | 11 | 16 | 16 | 19 | 20 |
| 7                                  | 5   | 4  | 14 | 18 | 18 | 19 | 20 |

The authors are grateful to Professor R. K. Sharan, Professor Y. L. Nene and Professor J. P. Sinha, of Patna, Pantnagar and Ranchi Universities, respectively, for their critical evaluation and the facilities extended.

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# NOTE ON THE OBSERVATION OF THE FUNGUS *GONGRONELLA BUTLERI* (LINDN.) PEYRONEL AND DALVESCO IN COCONUT ROOT

WHILE examining the longitudinal sections of coconut roots in connection with the investigations on the root (wilt) disease, it was observed that the

vascular tissue of the coconut root contained sporangia-like bodies (Fig. 1). Attempts were made to culture the organism by different methods. Root tissue containing the sporangium as well as sporangia separated by mixing the root tissue in Waring blender and squeezing through cotton wool were plated on potato-dextrose agar medium containing yeast extract and thiamine. 0.1% streptomycin was added to suppress the bacterial growth.

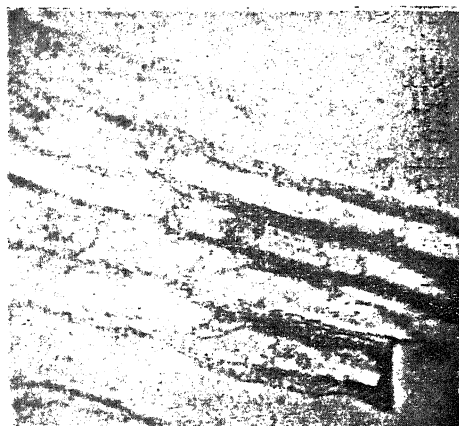


FIG. 1. Sporangium inside the cell.  $\times 125$ .

Fluffy growth with aerial mycelium was observed and the hyphae 2.0 to 3.5  $\mu$ m in width, were aseptate. Sporangium was apical with a diameter of 4 to 16  $\mu$ m. The fungus was identified as *Gongronella butleri* (Lindn.) Peyronel and Dalvesco belonging to the family Mortierellaceae of the order Mucorales under Phycomycetes. This appears to be the first report in coconut from India and is an addition to the list of fungi occurring in South India (Rangaswamy *et al.*, 1970). Further study to find out the association of the fungus, if any, with the root (wilt) disease complex is in progress.

Thanks are due to the Director, Commonwealth Mycological Institute, Kew, England, for the identification of the fungus and to Dr. K. Radha and Dr. P. Shanta, of the Central Plantation Crops Research Institute, Regional Station, Kayangulam, for suggestions in this line of work.

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# STUDIES ON GROWTH INHIBITION OF *FUSARIUM MONILIFORME* SHELDON BY SOME METALLIC CATIONS AND ANION IN CULTURE

INTEREST in the fungistatic activity of ions of heavy metals have developed from the attempts to control fungal pathogens<sup>1</sup>. Such fungistatic action has been attributed to a disruption of cellular metabolism

then incubated at 30° C in the dark. Three replicates were made for each concentration. Control sets (without the chemicals) were maintained under identical conditions. Mycelial growth at different concentrations was measured at 3, 6 and 9 days of the growth.

The differential growth has been recorded in Table I.

TABLE I

Effect of NaCN, ZnSO<sub>4</sub>, CuSO<sub>4</sub> and Co (NO<sub>3</sub>)<sub>2</sub> on the mycelial growth of *Fusarium moniliforme* Sheldon after 3, 6 and 9 days

| Concentrations of<br>the chemicals<br>(Molar) | Growth in diameter of the mycelia (cm)* |        |        |                   |        |        |                   |        |        |                                   |        |        |
|---|---|--------|--------|-------------------|--------|--------|-------------------|--------|--------|-----------------------------------|--------|--------|
|   | NaCN                                    |        |        | ZnSO <sub>4</sub> |        |        | CuSO <sub>4</sub> |        |        | CO(NO <sub>3</sub> ) <sub>2</sub> |        |        |
|   | 3 days                                  | 6 days | 9 days | 3 days            | 6 days | 9 days | 3 days            | 6 days | 9 days | 3 days                            | 6 days | 9 days |
| Control                                       | 2.6                                     | 5.0    | 8.6    | 3.0               | 5.7    | 6.5    | 2.6               | 5.0    | 8.0    | 3.3                               | 6.0    | 8.0    |
| 1 (M)   | 0                                       | 0      | 0      | 0                 | 0      | 0      | 0                 | 0      | 0      | 0                                 | 0      | 0      |
| 0.5 (M)                                       | 0                                       | 0      | 0      | 0                 | 0      | 0      | 0                 | 0      | 0      | 0                                 | 0      | 0      |
| 10 <sup>-1</sup> (M)                          | 0                                       | 0      | 0      | 0                 | 0      | 0      | 0                 | 0      | 0      | 0                                 | 0      | 0      |
| 10 <sup>-2</sup> (M)                          | 1.2                                     | 2.4    | 6.9    | 1.3               | 2.1    | 2.4    | 0                 | 0      | 0      | 0                                 | 0      | 0      |
| 10 <sup>-3</sup> (M)                          | 1.3                                     | 2.5    | 8.0    | 2.4               | 4.8    | 7.2    | 0                 | 0      | 0      | 2.1                               | 4.3    | 6.9    |
| 10 <sup>-4</sup> (M)                          | 1.4                                     | 2.8    | 7.7    | 2.6               | 5.2    | 6.3    | 2.8               | 5.4    | 8.3    | 3.0                               | 5.7    | 8.0    |
| 10 <sup>-5</sup> (M)                          | 1.6                                     | 2.8    | 7.9    | 2.7               | 5.2    | 6.7    | 2.9               | 5.3    | 8.5    | 3.1                               | 6.0    | 7.9    |
| 10 <sup>-6</sup> (M)                          | 1.6                                     | 3.5    | 7.7    | 2.8               | 5.2    | 6.6    | 2.9               | 5.3    | 8.4    | 3.2                               | 6.1    | 8.9    |
| 10 <sup>-7</sup> (M)                          | 1.5                                     | 3.0    | 7.6    | 2.9               | 5.7    | 7.0    | 2.9               | 5.7    | 8.7    | 3.2                               | 6.2    | 8.7    |

\* Average of three replicates.

due mostly to non-specific inhibition of a wide range of enzymes and co-enzymes particularly those dependent on sulphhydryl groups. The present investigation has been designed to study the metabolic inhibition by some metallic cations and the cyanide anion as indicated by growth inhibition of *Fusarium moniliforme* Sheldon in culture with a view to suggesting an effective fungicide for controlling the organism.

The test-fungus, *Fusarium moniliforme*, was isolated from malformations of mango inflorescence collected from Burdwan, West Bengal, 1972. Different concentrations of NaCN, ZnSO<sub>4</sub>, CuSO<sub>4</sub> and Co(NO<sub>3</sub>)<sub>2</sub> were mixed with Czapek's synthetic agar media and adjusted to the optimum pH 6.4. The substances selected in the present investigation are known metabolic inhibitors for micro-organisms. The relative tolerance of the pathogen against the substance can be effectively employed in combating the disease through growth inhibition. The media were sterilized, plated and inoculated finally at the centre with mycelia from the test-fungus grown on Czapek's synthetic agar medium. The samples were

It is evident from the result that Zn, Cu, Co and cyanide ions have distinct inhibitory effect on growth of the test-fungus. Growth is completely inhibited in Cu, Co, Zn and cyanide at concentrations above 10<sup>-4</sup>, 10<sup>-3</sup>, 10<sup>-2</sup>, 10<sup>-2</sup> molar respectively revealing thereby that Cu is the most toxic one among the three metallic cations followed by Co and Zn. This is in full conformity with the previous observations. Toxic actions of cations and the anion are considered to act as inhibitors by inactivating the enzymes. All the metallic cations have, however, been found to promote growth at very low concentrations (10<sup>-7</sup>) revealing thereby their importance as micronutrients.

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# A NEW GENE IN SOYBEAN [*GLYCINE MAX* (L.) MERR.] CONDITIONING HYPOCOTYL PIGMENTATION\*

JOHNSON AND BERNARD (1962) have listed all the genes identified in soybean [*Glycine max* (L.) Merr.] controlling qualitative characters till that date. No gene is, however, listed there which controls hypocotyl pigmentation in soybean and neither it is reported yet.

mented and non-pigmented parents were studied for pigmentation of hypocotyls in  $F_1$  generation and the  $F_2$  segregation pattern was recorded. Each of these five crosses, considered for the study, was treated as separate family and the  $\chi^2$  for segregation was calculated separately. The  $\chi^2$ s for deviation and heterogeneity were computed. The results of this analysis and the respective  $F_1$  characteristics are given in Table I.

TABLE I

| Crosses           | Hypocotyl<br>coloura-<br>tion of F <sub>1</sub> | F <sub>2</sub> segregation for hypocotyl colouration |        | $\chi^2$<br>(3: 1) | P<br>lies between |
|-------------------|---|--|--------|--------------------|-------------------|
|                   |   | Pigmented  | Green  |                    |                   |
| 1                 | 2   | 3 (a)  | 3 (b)  | 4                  | 5                 |
| Lee × Masterpiece | Pigmented                                       | Obs. 687   | 241    | .4654              | .50-.30           |
|                   |   | Exp. 696   | 232    |                    |                   |
| Lee × Bragg       | Pigmented                                       | Obs. 231   | 82     | .2396              | .70-.50           |
|                   |   | Exp. 234.75  | 78.25  |                    |                   |
| Lee × Hill        | Pigmented                                       | Obs. 334   | 119    | .3892              | .70-.50           |
|                   |   | Exp. 339.75  | 113.25 |                    |                   |
| Lee × Hardee      | Pigmented                                       | Obs. 192   | 68     | .1845              | .70-.50           |
|                   |   | Exp. 195   | 65     |                    |                   |
| Amsoy × Telstar   | Pigmented                                       | Obs. 427   | 149    | .2314              | .70-.50           |
|                   |   | Exp. 432   | 144    |                    |                   |
|                   |   | $\chi^2$   | d.f.   | P                  |                   |
| Deviation         |   | .4810  | 1      | .5-.3              |                   |
| Heterogeneity     |   | 1.0292   | 4      | .95-.90            |                   |

In the course of studying several crosses made with objectives other than genetical studies alone, some of the heterozygotes were found to segregate for the hypocotyl colouration. The pigmentation in soybean appears on germination and emergence of the hypocotyl above ground and persists till the plant ages. On the other hand, the non-pigmented types do not form any pigment at all on germination and neither on aging.

Of the seven parents involved in the various crosses two, viz., Lee and Amsoy produced pigmented hypocotyls while five, viz., Masterpiece, Bragg, Hill, Hardee and Telstar produced green hypocotyls. Five sets of crosses involving pig-

Considered individually, in each of the crosses a 3 pigmented : 1 green ratio fitted very well showing complete dominance of the pigmented character in  $F_1$ . The  $\chi^2$  for deviation also indicated that this ratio fitted well when all the crosses were considered together. The result of  $\chi^2$  analysis for heterogeneity indicated clearly that all the crosses fitted well to 3 : 1 ratio.

Thus the character pigmented and green hypocotyl colouration appear to be governed by a single gene. A symbolisation of  $Hy$ , is suggested for the gene conditioning pigmented character of the hypocotyl. Soybean being a difficult material for the purpose of crossing because of its having very

small sized flowers, a breeder would like to be sure of the genuineness of the crosses he takes up. This character of hypocotyl pigmentation can be advantageously utilised as a marker gene.

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\* The work was carried out at I.A.R.I. Vegetable Research Station, Katrain, Kulu Valley.

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#### NOTE ON THE DEVELOPMENT OF VESICULAR-ARBUSCULAR MYCORRHIZA— *ENDOGONE FASCICULATA* IN COCONUT ROOT

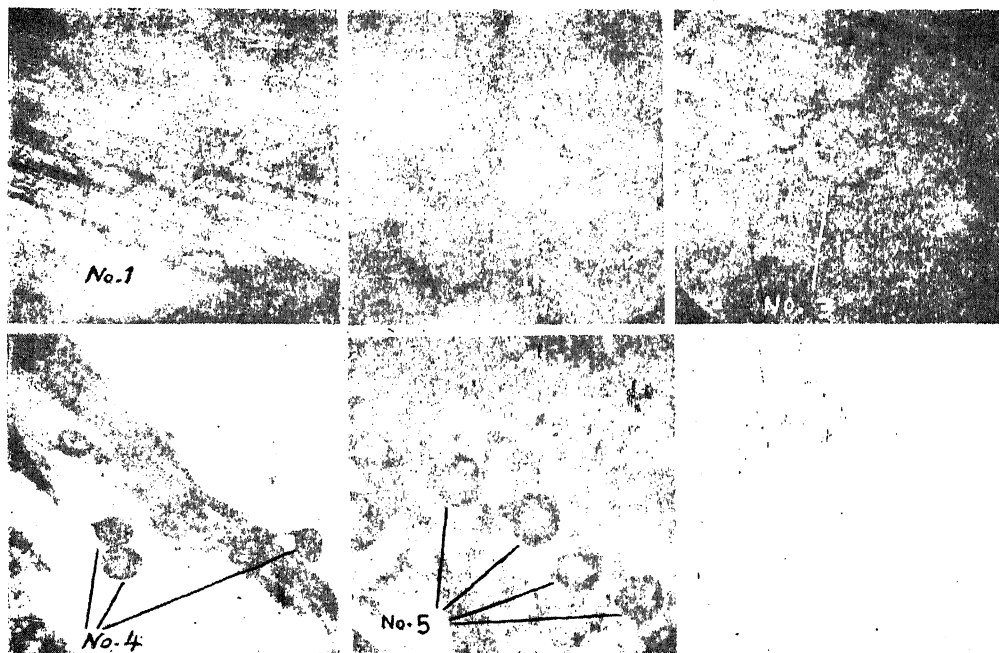
OF the various mycorrhizas reported the most widespread is the so-called vesicular-arbuscular type (Nicolson, 1967). Gerdemann (1968) made a review on this and later more than hundred papers have been published on this aspect, reviewed by Mosse (1973). The long standing speculation about the identity of vesicular-arbuscular endophytes (Gerdemann, 1968) has largely been resolved in favour of one or another species of *Endogone*. Improvement in growth and also the uptake of

increased phosphate was observed in many plants having micorrhizal association (Mosse, 1973). Occurrence of the vesicular-arbuscular mycorrhiza was noted in the roots of healthy and diseased coconut palm while studying the lower form of fungi associated with coconut root.

Coconut root materials were collected from different places in Kerala. Longitudinal sections of these were stained by boiling for one minute in acid fuchsin-lacto-phenol, destained and mounted in clear lacto-phenol for microscopic examinations. The hyphae on the root surface were broad with globules inside, measured 12 to 16  $\mu$  in width whereas the hyphae in the inner cortical cells measured 2 to 8  $\mu$  in width. The mycelium on the surface as well as inner cells appeared swollen at the apical portion to form vesicles ranging in size from 40  $\mu$  to 100  $\mu$  (Fig. 1). Dark thick-walled vesicles were also seen on the surface of the root. This was identified as *Endogone fasciculata*. This appears to be the first report on coconut.

This fungus was also observed in the roots of *Cassia tora*, *Melothria* sp., *Phyllanthus neuri*, *Solanum nigrum*, *Leucas aspera*, *Mullugo* sp., *Physalis minima*, etc., common weeds growing in coconut gardens.

Grateful thanks are due to Dr. Harley, Professor, Forest Science, Oxford University and Dr. T. H.



FIGS. 1-5. Fig. 1. Vesicle in the tender root of coconut,  $\times 125$ . Fig. 2. Vesicle in clusters in the tender root of coconut,  $\times 125$ . Fig. 3. Single vesicle with aseptate mycelium,  $\times 400$ . Fig. 4. Vesicles in mature root of coconut,  $\times 125$ . Fig. 5. Thick-walled spores in mature root of coconut,  $\times 125$ .

Nicolson, Professor, Department of Biological Science, the University of Dundee, for identification of the fungus. Thanks are also due to Dr. K. Radha and Dr. P. Shanta, of the CPCRI, Regional Station, Kayangulam, for the suggestions given in this study. CPCRI, Regional Station, V. G. LILY. Kayangulam, Krishnapuram 690533, Kera'a, July 1, 1974.

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#### A NEW LEAF BLIGHT OF *CLERODENDRON FRAGRANS*, R. BR.

A SEVERE leaf blight disease of *Clerodendron fragrans*, R. Br., a roadside and a popular ornamental plant, was observed during the summer season of 1973 and 1974 around Madanapalle, Chittoor District, Andhra Pradesh. The disease manifests itself in the form of irregular grey brown necrotic areas or patches measuring 4–14 mm long in size. The disease is very characteristic in that the patches appear or begin mostly along the margins and tips of the leaves. These areas gradually extend downwards along the margin involving the major part of the leaf tissue, ultimately resulting in blight (Fig. 1). In cases of severe infection, the plants

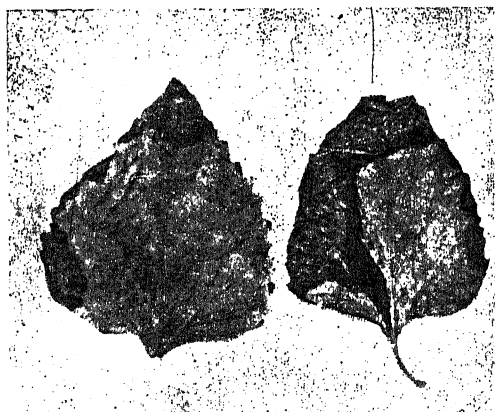


FIG. 1

look withered and could be spotted from a distance. Individual spots appear very rarely in the middle of the leaves. Severe attack results in complete drying of the leaves and occasional defoliation. The young as well as mature leaves were seen to be equally attacked.

The fungus was isolated by plating on PDA medium and all the single spore isolations made were found to be identical. The fungus was established in pure culture on PDA and its pathogenicity was proved by spraying spore suspension (prepared in sterile water from one week old culture) on the leaves of around one month old healthy plants. Slight injuries were made over some of the leaves with a sterile needle before inoculation. Control plants were sprayed with sterile water only. The inoculated plants were kept inside a humid chamber for 48 hours. Typical blight symptoms developed in 10–12 days in both the injured and uninjured leaves but none in the control. Re-isolations yielded the original fungus.

Aerial mycelium bluish green, cottony, abundant and appearing somewhat powdery with conidial formation and produced dark blue pigmentation on the medium. The hyphae septate, branched and measured 3–4.5  $\mu$  in width. On the leaf, the pathogen produced conidiophores and conidia. Conidiophores light to dark brown simple or branched having distinct geniculations. Conidia yellowish brown, obclavate, smooth walled 2–7 (3–8 celled), transverse and 1–2 longitudinal septa with or without beak. The length of the conidia varies (with beak) from 21 to 45  $\mu$  and width 3 to 15  $\mu$ .

The causal organism has been identified and confirmed as *Alternaria* state of *Pleospora infectoria* Fuckel. (IMI 184579).

Grateful thanks are due to Dr. Ellis of CMI Ferry Lane, Kew, England, for identification of the fungus and to Prof. V. S. R. Das for providing necessary facilities.

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#### CYTOLOGICAL STUDY OF A POLYPLOID O RADISH OBTAINED AS A RESULT OF INBREEDING

ALMOST all the inbred lines of radish (*Raphanus sativus* L. var. *radicola* Pers.) in the genetic collection of Dr. S. I. Narbut of the Chair of Genetics and Plant Breeding, Leningrad State University, Leningrad (USSR), significantly differ in many characters from their original populations and are generally characterized by reduced fertility<sup>1</sup>. One of the lines, namely, LB-274, which was isolated

from the Russian variety "Virovsky bellie", was found to be highly uniform and homozygous with no further segregation in the 8th generation of inbreeding. In contrast to population, its plants had practically no anthocyanin pigments in the stem and leaves and the flowers were dark-violet in colour. In the year 1971, i.e., in the 15th generation of inbreeding, two of its plants appeared to differ from the rest in the size of the leaves, flowers and growth. They were not only completely self-sterile but also produced no seed at all even under panmixis. These plants were suspected to be autotetraploids. A cytological investigation was, therefore, undertaken to see whether these plants were polyploids or not.

For cytological analysis flower buds from suspected polyploid plants as well as the plants of the inbred line, LB-274, were fixed in the Carnoy's solution for six hours. The anthers were squashed in 1% acetocarmine and examined. 50 pollen mother cells were scored in each case.

The plants of the inbred line, LB-274, showed normal meiotic division, forming 9 bivalents at Metaphase-I. The number of rod bivalents, however, varied from one to three per cell. But quite a different picture was found in the plants, suspected to be tetraploids. In them all the p.m.c. were polyploids with various types of configurations known for a typical autotetraploid. No diploid cell was observed. An analysis of 50 cells at meiosis gave the following results :

| Quadri-valents | Trivalents | Bivalents | Uni-valents | No. of cells |
|----------------|------------|-----------|-------------|--------------|
| 3              | 3          | 5         | 5           | 15           |
| 2              | 5          | 4         | 5           | 12           |
| 1              | 7          | 4         | 3           | 9            |
| 0              | 6          | 5         | 8           | 6            |
| 5              | 2          | 2         | 6           | 5            |
| 2              | 4          | 3         | 10          | 3            |

Their complete sterility may be attributed to highly irregular meiosis and self-incompatibility.

Presence of polyploid cells in the inbred lines of allogamous populations is not new. It has been reported in rye<sup>2-3</sup>. Rees<sup>4</sup> has noted 1% polyploid cells in an inbred line of rye. Such cells have also been observed in one of the inbred lines of radish, LS-337/25<sup>5</sup>. From these, errors at the pre-meiotic mitoses have been inferred in the homozygotes. It seems that in the present case pre-meiotic errors have led to the formation of unreduced diploid gametes which on fusion gave rise to polyploid plants.

The author wishes to record his sincere thanks to Dr. S. I. Narbut of the Chair of Genetics and Plant Breeding, Leningrad State University, Leningrad, for the material.

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### PEMPHIGUS BURSARIUS LINN. PRODUCING GALLS ON LOMBARDY POPLAR IN KASHMIR

THE Lombardy Poplar, *Populus nigra italica* Muenchh., is commonly grown in Kashmir on the roadsides and is a graceful avenue tree. The author collected a large number of pear-shaped or irregularly purse-shaped galls formed at various positions on the leaf stalks (Fig. 1). The causative agent was subsequently identified as *Pemphigus bursarius* (L.) (Homoptera : Aphididae). These galls were particularly more numerous on the lower leaves. On dissection each gall was found occupied by a large sized fundatrix, a considerable number of alate females, numerous young ones belonging to various developmental stages, honey-dew and cast skins entangled in a white powdery matter.

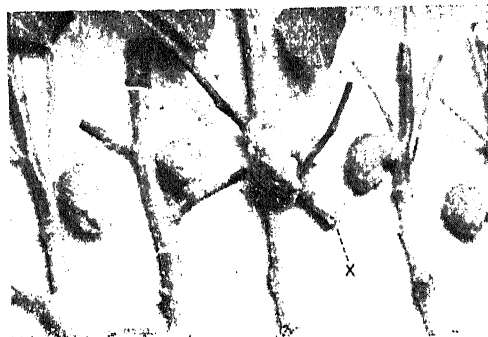


FIG. 1. Galls of *Pemphigus bursarius* (L.) on Lombardy Poplar. (X) Beak-like escape vent in the gall.

The gall formation gets initiated in early spring when a fundatrix hatching from an over-wintered egg pierces the petiole of an unfolding leaf to suck the sap. The plant cells at this region multiply so as to give rise to a rather lopsided purse-like structure enclosing the fundatrix. The fundatrix reproduces asexually within the gall to give rise to a generation of alate viviparous females which

escape from the gall in late summer particularly during August, when most of such galls are found empty. Populations of this aphid were detected on younger shoots of Lombardy Poplars in early autumn, and were found to consist of both males and females. It could not be ascertained in the preliminary observations whether these aphids had dispersed to the younger shoots after escaping from the mature galls, or had meanwhile migrated to the roots of various Compositae which serve as the secondary hosts for this aphid elsewhere<sup>1</sup>, before returning to the Lombardy Poplars in early autumn. The autumn generation reproduces sexually and the eggs over-winter on the young twigs. This aphid appeared to be exclusively restricted to the Lombardy Poplars and was not recovered from any other *Populus* species in Kashmir. The average sized galls measured 1.5–2.0 cm. This is the first report of *Pemphigus bursarius* (L.) from Kashmir. Govt. Agriculture College, M. ZAKA-UR-RAB. Sopore (Wadura), Kashmir, August 12, 1974.

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### STUDIES IN GERANIALES

#### III. The Structure and the Development of the Fruit Wall in *Averrhoa carambola* L.

*Averrhoa carambola*, a member of the family Oxalidaceae, is a moderate sized tree, with minute rose-purple flowers, arranged in axillary cymes, often forming panicles. The fruit is a five or six angled berry with acute lobes and arillate seeds. Since these fruits are rich in acid juice, they are eaten raw, cooked or preserved, and are prescribed as antiscorbutics. The structure and the development of the fruit wall has not yet been worked out in this interesting genus.

The fruits of different ages, collected in August 1972 from plants growing locally, were preserved in 70% alcohol. The material was dehydrate by passing through tertiary butyl alcohol grades and embedded in paraffin wax. The study was based on transverse sections of the fruits of different ages, varying in length from 3 mm to 40 mm and stained in Safranin-Fast green.

In a T.S., the pericarp of a young fruit (3 mm) is 14 or 15 cells thick, the cells being compact and parenchymatous. The cells of the outer epidermis are more or less rectangular and those of the inner epidermis are tangentially elongated. The layer, lying immediately next to the inner epidermis also has cells which are somewhat tangentially elongated. The cells are richly cytoplasmic and undergo both anticlinal and periclinal divisions. At this stage a

few tannin cells are found interspersed in the pericarp (Fig. 1).



FIGS. 1–4. *Averrhoa carambola* L. Some stages in the development of fruit wall as understood from transverse sections. Fig. 1. From fruit about 3 mm in length,  $\times 260$ . Fig. 2. From fruit 6–8 mm in length,  $\times 60$ . Fig. 3. From fruit 12–15 mm in length,  $\times 20$ . Fig. 4. From fruit about 40 mm in length,  $\times 20$ .

The cell divisions continue until the fruit attains a length of about 6–8 mm. At this stage the pericarp may be distinguished into three more or less distinct zones: (i) exocarp or epicarp consisting of a few layers of small, more or less cubical cells; (ii) mesocarp that constitutes the main bulk of the pericarp with several layers of irregular cells and (iii) endocarp with elongated cells of 4 or 5 layers including the inner epidermis. The development of tannin is maximum at this stage (Fig. 2).

In a fruit measuring 12–15 mm in length cell division ceases to occur and the further growth is entirely by cell enlargement, especially in the mesocarp. Tannin also disappears by this time and the cells of the endocarp become compressed by the enlarging cells of the mesocarp (Fig. 3).

The subsequent increase in the size of the fruit is by cell enlargement in the region of the mesocarp. In a fruit about 40 mm in length, the enlargement of the cells is so vigorous that the cell walls

disintegrate. At this stage certain groups of small cells are also seen in the mesocarp in between the enlarged cells that are filled with juice. These groups of small cells are formed due to the pressure of the surrounding enlarging cells (Fig. 4).

The author takes this opportunity to record his sincere thanks to Professor V. Puri, Meerut University, Meerut and to Dr. Virendra Singh, Meerut College, Meerut, for their valuable suggestions.

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### NEUTRON INDUCED VARIEGATED MUTATION IN TAPIOCA

THIS is a report on a variegated mutant obtained in the neutron irradiated population of tapioca.

Fresh cuttings of a promising tapioca (*Manihot esculenta* Crantz) var. *H-97* (obtained from Dr. N. Hrish, Director, Central Tuber Crops Research Institute, Trivandrum) were exposed to fast neutrons using Standard Neutron Irradiation Facility (SNIF) in the APSARA Reactor of the Bhabha Atomic Research Centre. The dose rate was 71 rad/min and 12 cuttings were exposed per treatment. The radiation dose ranged from 25 to 250 rad. The cuttings along with untreated control were planted in the field immediately after treatment.

One of the two sprouts developing from one cutting in 125 rad treatment was a sectorial chimera with variegated leaves in early growth period in the  $MV_1$  generation. After harvest, the stem which had variegated leaves was made into ten cuttings and planted. In the  $MV_2$  generation, two sprouts in the population had all their leaves variegated; the lobes were light green with white border (Fig. 1). Three other sprouts in early stages of growth were chimeric and had variegated leaves as in the  $MV_1$  generation. One of these sprouts later developed normal green leaves while in the other two, the leaves later developed were variegated. All the other sprouts in the population were normal green.

There is a garden variety of tapioca (*M. esculenta* Crantz var. *variegata* Hort.) which is having variegated leaves with yellow middle region and green border. The variant obtained in the present study is conspicuously different from the existing variegated type with respect to chlorophyll distribution and its nature. On account of low chlorophyll content, the growth was less vigorous in the variegated mutant. As the palisade cells did not possess chlorophyll and were mostly empty the leaves appeared light green especially on the ventral side.

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### PROTEIN CONTENT IN HEALTHY AND YELLOW MOSAIC INFECTED SOYBEAN SEEDS

DURING a survey of important soybean growing areas of Uttar Pradesh in 1971 and 1972 soybean was found infected with yellow mosaic everywhere, except in the hills. Its incidence varied from 10-30% in different areas. The samples from these areas were collected and studied at University of Gorakhpur. The effect of yellow mosaic disease on protein content of seeds of some important soybean varieties was studied in the present paper.

Four varieties of soybean, viz., Bragg, Clark-63, Lee and Local-2 were selected. For each variety two lots each of 20 seedlings were taken. One lot was treated with white flies (*Bemisia tabaci* Gennadius) already fed on the diseased leaves. The other lot was left healthy. The plants were kept in insect proof conditions. When they attained maturity the seeds were collected separately and subjected to protein estimation which was done by the following method.

One hundred mg of dried seeds were crushed with 10 ml of trichloroacetic acid. This was then centrifuged at 1600 rpm. To the residue 1 ml of digestion mixture (2.4 g of selenium/litre of conc.  $H_2SO_4$ ) was added. It was mixed well and allowed

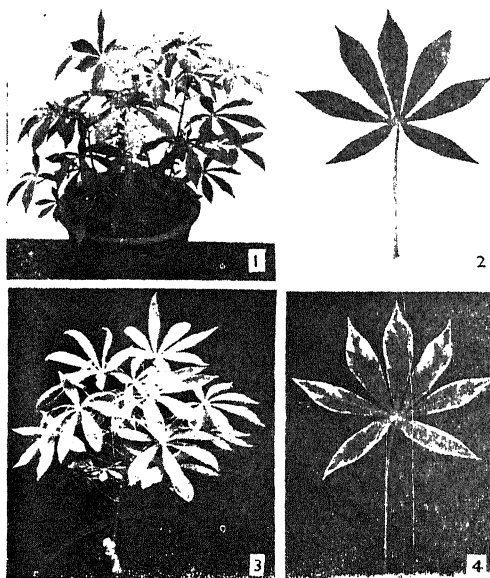


FIG. 1. Tapioca var. *H-97* and the variegated mutant. A and B—Plant and leaf of parent *H-97*. C and D—Plant and leaf of the Variegated mutant.

to stand for 30 minutes. Four drops of 50% sodium thiosulphate solution followed by 5 ml of another digestion mixture (32 g salicylic acid/litre of conc.  $H_2SO_4$ ) were then added. After the digestion was complete, about 5 drops of 10% perchloric acid was added and the contents were heated slowly till the solution became clear. The solution was then made up to 100 ml. In a colorimetric tube 1 ml of this solution, 8.5 ml of Nessler's reagent and 0.5 ml of gum-ghati solution were added. The contents were mixed thoroughly. The ammonia thus evolved was estimated in 'A.M.I.L.' Biochem. Absorptiometer using filter No. 42.

The value of organic nitrogen thus obtained was then multiplied by a factor 6.25 to obtain total protein content.

The results given in Table I show that yellow mosaic increased the protein content in the seeds in all the four varieties tested. Maximum increase was recorded in the variety Local-2 and it was least in Bragg. The increase was dependent upon the susceptibility of the varieties the most susceptible variety having the maximum protein.

TABLE I

*Protein content in healthy and yellow mosaic infected seeds*

| Varieties | % protein in the healthy seed | % protein in the diseased seed | % increase |
|-----------|-------------------------------|--------------------------------|------------|
| Bragg     | 40.6                          | 43.0                           | 2.2        |
| Clark-63  | 42.5                          | 45.5                           | 3.0        |
| Lee       | 41.2                          | 46.7                           | 5.6        |
| Local-2   | 36.3                          | 42.5                           | 6.9        |

Increased protein content in virus affected plant parts has been reported by some workers<sup>1-6</sup> while some others have reported a reduction in protein content due to virus disease<sup>1,7</sup>.

In the present study protein content of the seeds is increased in all the varieties of soybean affected by yellow mosaic. This increase appears to be due to the increased amount of free amino acids and total nitrogen which have led to an increased rate of protein synthesis through condensation of amino acids.

The authors are thankful to Dr. K. S. Bhargava for providing necessary laboratory and library facilities. They are also grateful to Dr. R. D. Joshi for his helpful suggestions.

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#### A NEW LEAF SPOT DISEASE OF *CENTELLA ASIATICA* L.

A SEVERE leaf spot disease of *Centella asiatica* L. was observed during summer season around Madanapalle in Chittoor District. The disease manifests itself in the form of leaf spots which are elongated or circular, yellowish brown when young and becoming dark brown with age, with a greyish white centre bordered by deep brown margin. In cases where the infection starts from the apex of the leaf the patch may extend and cover about half of the lamina (Fig. 1).

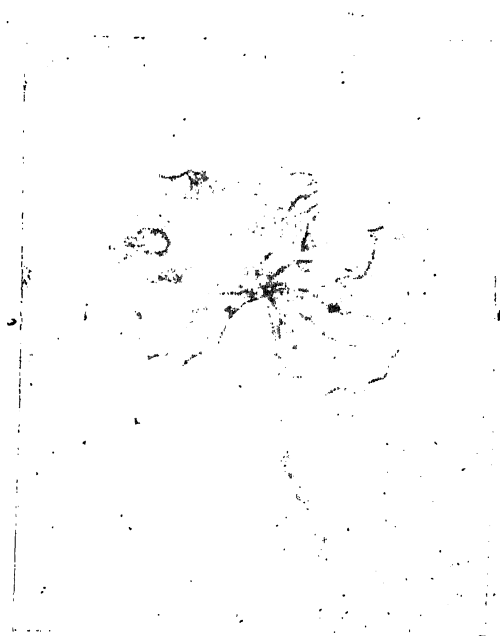


FIG. 1

The fungus was isolated in pure culture from the necrotic spots of the leaves and on inoculation to healthy leaves proved to be a virulent pathogen. The organism consistently produced the typical symptoms and its morphological and cultural characteristics were exactly similar to the previous isolate.

The fungus grew and sporulated well on PDA medium. Mycelium grey coloured with green pigment deposited on the medium. The hyphae septate, branched and measured  $3-6\mu$  in width. Only conidiophores and conidia were produced. Conidiophores dark brown, unbranched and erect. Conidia clavate, slightly bent or curved, rarely triangular with flattened ends,  $2-4$  celled ( $1-3$  septate), light brown in colour and measured  $12$  to  $21 \times 2$  to  $9\mu$ . One or two of the middle cells were disproportionately enlarged and more darkly coloured than the end cells.

The causal agent has been identified and confirmed as the *Curvularia* state of *Cochliobolus geniculatus* Nelson (IMI 184580).

Thanks are due to Dr. M. B. Ellis, Commonwealth Mycological Institute, Kew, England, for identifying the pathogen and to Prof. V. S. R. Das for providing facilities.

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### MEALY BUGS ON THE ROOTS OF *PARTHENIUM* WEED

*Parthenium hysterophorus* Linn., commonly called "congress grass" is at present an aggressive weed in many parts of India. It is not known to be affected by any pest or disease<sup>1</sup>. Recently, Anupam Varma *et al.*<sup>2</sup> have reported mycoplasmal etiology for this plant growing at Delhi and proposed its utility for the biological control. In some plants

of the same species growing at Mysore City, attack of mealy bugs was observed on their roots. Young plants were especially prone to the attack and later they died. Such plants were seen wilted. The nymphs and adult females lodge on the root surface and feed on them (Fig. 1). The bug is identified

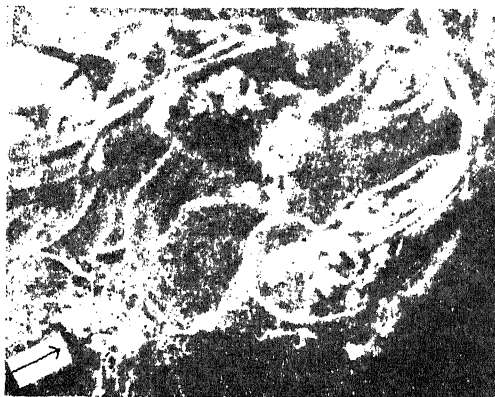


FIG. 1. Roots of *Parthenium* infected with mealy bugs.

as *Ferrisia virgata* Cockerell. Further search for other pests attacking the plant is essential for finding out their use in biological control of this pernicious weed.

We thank Mrs. L. Huddleston, British Museum (Natural History), London and Dr. G. P. Chenna-Basavanna, Professor of Entomology, UAS, Bangalore, for identification of the mealy bug.

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## SHORT SCIENTIFIC NOTES

### Discovery of Phosphorite in the Palaeocene Eocene Rocks of Northwestern Himalayas

During the course of investigations of the project "Prospecting of Phosphorite in Northwestern Himalayas", the authors discovered a number of phosphate bearing horizons in the Palaeocene-Eocene sediments exposed in the vicinity of Nahan (Sirmur District, Himachal Pradesh). Concentration of phosphatic material is observed in olive green shales, brownish green siltstones and pale brown limestones. Dark grey coloured phosphatic nodules having diameter up to 10 cm occur in crushed carbonaceous matter. The phosphatic nodules assay as high as 26.16%  $P_2O_5$ . The phosphatic material occurs in the form of pellets ranging in size from minute microscopic to over 10 mm, void filling, intimately intermixed with matrix and as nodules. The phosphate bearing beds extend in the strike direction for a considerable length and warrant serious attention.

The only known occurrence of phosphatic material in the Palaeocene-Eocene rocks of Himachal Pradesh is from Mahasu District (Aggarwal<sup>1,2</sup>; Chaudhri<sup>3</sup>). Detailed work is in progress.

Centre of Advanced Study in Geology, Punjab University, Chandigarh,  
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### *Neanotis montholoni* (Hook. f.) W. H. Lewis: A New Record for Andhra Pradesh

*Neanotis montholoni* (Hook. f.) W. H. Lewis, (Rubiaceae) was first collected in September, 1968 from Kamareddy, Andhra Pradesh. It was identified by the Royal Botanic Gardens, Kew and deposited there. Duplicates are deposited with (CAL.) and (BLATT.) under voucher No. Bahadur 105.

The species hitherto known to occur, only in the South-West Indian hills is an addition to the flora of Andhra Pradesh. This collection from a place far from its original place of collection, i.e., Concan and Southwards (HK. f., *Fl. Brit. India*, 3 : 73, 1880), Mysore and Canara to Malabar (Gamble, *Fl. Madras*, 2 : 427, Rep. 1967), Concan, Poona, Belgaum (Cooke, *Fl. Bombay*, 2 : 22, Rep. 1967).

The present collection suggests the possibility of a very wide range of its distribution. Further, the species which is characteristic of higher elevations (2,600 m above sea level) is now being found at sea level. The following herbarium specimens have been examined which suggest wider distribution of this species in the plains of Maharashtra and the adjoining border districts of Andhra Pradesh where from Kamareddy is very close.

Ellichpur, Central Provinces and Berar, 15-12-1894, G. Watt, 15392 (CAL.), Bhausa, Narsinpur District, Plateau of Deccan, 13-8-1903, Kalka Pershad, 15393 (CAL.) Khandwa, Nimar District, 23-9-1908, I.H. Burkill, 31005 (CAL.), Nasik Road, Nazik District, 11-9-1910, D. Hooker, 3446 (CAL.).

The species flowers and fruits from August to November. The mode of dispersal and distribution is not known but could be due to wind or water as the seeds are light.

My grateful thanks to Sir George Taylor for providing the identification and to Dr. D. B. Deb for the loan of *Anolis* material and Prof. U. B. S. Swami for encouragement.

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### Studies on the Association between Azotobacter Population and Asymbiotic Nitrogen Fixation in Calcareous Soils of Pusa, Bihar

The present investigation was undertaken to study the association between azotobacter population and asymbiotic nitrogen fixation in calcareous soils.

The population of azotobacter was estimated by plate dilution method in Jensen's medium (Allen, 1957). With a view to establishing the gain in the amount of nitrogen, one gram of the soil was incubated in 100 ml of Ashby's mannite broth medium for 21 days and the amount of nitrogen fixed asymbiotically by azotobacter was estimated by Kjeldahl's method. A control with one gram of soil under similar condition was also run to calculate the gain in amount of nitrogen. The results have been shown in Table I.

The highest population of azotobacter was found in silty loam followed by sandy loam, silty clay loam and clay loam soils. The lowest count was noted in clayey soils. It will be further seen that

TABLE I

| Soil type          | Average<br>azoto-<br>bacter<br>count<br>(10 <sup>5</sup> ) | Amount of<br>nitrogen in mgm |         | Gain in<br>nitrogen<br>in mgm<br>per gram<br>soil |
|--------------------|--|------------------------------|---------|---|
|                    |  | Treat-<br>ed                 | Control |   |
| 1. Silty loam      | 22.58  | 8.02                         | 1.30    | 6.72  |
| 2. Sandy loam      | 15.83  | 6.61                         | 1.19    | 4.42  |
| 3. Salty clay loam | 14.80  | 4.95                         | 1.52    | 3.43  |
| 4. Clay loam       | 7.20   | 4.05                         | 0.97    | 3.07  |
| 5. Clay            | 5.66   | 4.02                         | 1.24    | 2.78  |

the gain in amount of nitrogen varied directly with azotobacter population, the correlation coefficient between counts of azotobacter in different soil types and amount of gain in nitrogen being positive and significant at less than 5% level ( $r = 0.899$ ).

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### Pythium Stalkrot—A Deadly Disease of Maize in India

Pythium stalkrot of maize incited by *Pythium aphanidermatum* (Edson) Fitzp. (= *P. butleri* Subr.) was first reported in India by Srivastava and Rao (1964) and after release of hybrids and composites, the disease appeared in epidemic proportions in certain parts of India.

The disease attacks the crop during July–August in Kharif season and its occurrence has been recorded from various locations in Delhi, Punjab, Haryana, Himachal Pradesh, Bihar, Uttar Pradesh and Andhra Pradesh. In 1969, the disease assumed epidemic proportions at Sundergarh (Himachal Pradesh) and the incidence was recorded upto 40% which severely damaged the crop.

The experiments conducted during 1967–71 at IARI, New Delhi, have revealed that the Pythium stalkrot of maize occurs when the vulnerable pretasseling stage of crop, coupled with high plant population per unit area in a poorly-drained field coincides, with high atmospheric temperature (30–35°C) and high relative humidity (90–100%) as prevalent in the month of August.

The following control measures are suggested to combat this menace:

- (i) Comparatively tolerant hybrids and composites like Him-123, Hi-starch and Vijay should be selected for planting.
- (ii) Regulation of planting time either before first week of June or after second week of July.
- (iii) Plant population should not to exceed 50,000 plants per hectare,
- (iv) Proper field drainage to be maintained to avoid waterlogging.
- (v) Previous crop residue or debris to be removed to avoid the chances of the buildup of the pathogen thereupon,
- (vi) Balanced fertilizer application at the rate of 120 kg nitrogen, 50 kg phosphorus and 50 kg potassium.
- (vii) Soil drenching with 2% solution of Captan at the rate of 1000 litres per acre at preflowering stage of crop,
- (viii) Excessive organic manuring not be resorted to.

The authors are thankful to Dr. S. P. Raychaudhary, Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-12, for providing the facilities to carry out these investigations.

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### Pathology of Chlamydial Abortions in Ovine and Caprine

A storm of late abortions in sheep and goats (77 sheep and 3 goats) alongwith premature birth of weak off-springs were proven to be due to chlamydial agent for the first time in India.

The majority of cases had necrosed placental cotyledon and acute exudative placentitis. Generalised congestion with enlargement of spleen and liver was present in some foetuses. Liver was most commonly involved having perivascular reticuloendothelial cell proliferations and degenerative foci. Presence of elementary bodies in the smears of placentae, foetal organs, thoracic and abdominal fluid was demonstrated by Macchiavello and Gimenez stains. These observations are in

conformity with the previous workers<sup>1,2</sup>. Gliosis in brain of aborted foetuses was an additional feature necessitating further studies.

In parallel with histo-anatomical studies isolation of the chlamydial agents in the yolk sac of embryonating chicken eggs confirmed the disease as chlamydiosis.

Complement fixing antibodies in 21 days post-abortion sheep sera were demonstrated with standard chlamydial group antigen and antigen prepared presently.

The immunofluorescence studies with the group specific conjugated globulin confirmed these organisms in the sections of cotyledon and yolk sac membrane as Chlamydia.

Authors thank Dr. J. Storz and Dr. L. Blanco for supplying the antigen and group specific conjugate. The facilities provided by Dr. C. M. Singh, Director of this Institute are also acknowledged.

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### Occurrence of Spontaneous Haploid in *Ricinus communis* L. var. Aruna

Haploid has been reported in castor—*Ricinus communis* L.— by Poole and Hadley<sup>1</sup> from selfed seed of P.I. 183468. There are reports of haploids in other crop plants reviewed by Kimbar and Riley<sup>2</sup> and Magoon and Khanna<sup>3</sup>. Since 1954 there has been no report about haploid in castor and the work developed from such a plant in developing homozygous diploid line. Present paper deals with the haploid obtained in *Ricinus communis* variety Aruna.

In Aruna at Regional Research Station, Raichur, India, an interesting plant was observed. This plant was dwarf with a few rough and narrow leaves and

produced spike under field conditions which did not set any seed. The plant was dug out and potted in the bigger pot. Transplanting had enormous effect on the plant by way of producing plenty of foliage and production of spikes in addition to the primary ones. Cytology of the plant revealed in root tip preparations following oxyquinoline technique that it possessed ten somatic chromosomes as compared to the twenty that are seen in the normal plants. Flower buds were used for meiotic study which also showed ten univalents. Spikes produced from the haploid plant possessed shrivelled seeds. In order to develop homozygous diploid from the haploid, the secondary branches have been treated with various concentrations of aqueous colchicine (0.1 to 0.5%) to develop inbred lines for use in commercial hybrid seed production. However the attempt was not successful in obtaining homozygous diploids. Studies in castor show that the utilisation of heterosis seems to have limited scope in hybrids of the varieties HC 6, Gujarath Monospoke, Cimmarron Inbred, Aruna, Rosy and TMV 11. However the development of homozygous diploid lines as is envisaged might help in developing commercial hybrids. The said haploid is surviving showing perennial habit. Attempt of diploidizing has been continued.

The authors take the opportunity of thanking Dr. J. V. Goud and Dr. N. B. Kajjari for encouragement and providing facilities.

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## ABSTRACT

The fossil fission track technique has been used in dating Precambrian muscovite samples of Bihar Mica Belt (Kodarma, District Hazaribag). The average age obtained for this belt is  $865 \pm 72$  million years which agrees with the age determined by radiometric methods.

## INTRODUCTION

**T**HE fossil fission track technique was introduced by Price and Walker (1963) for dating of rocks and mineral samples of geological origin. It utilises the fact that uranium is present in almost all the minerals and it undergoes spontaneous fission at a rate much slower than its usual disintegration through  $\alpha$ -decay. The fission fragments which are produced travel inside the mineral leaving their tracks as radiation damaged regions. These tracks are of thickness 100 Å and can be directly observed under an electron microscope<sup>1</sup>. However, they can be observed under an optical microscope<sup>2</sup> by etching the tracks with suitable chemical reagents.

**Theory.**—The formula used for calculating the age of the samples of Precambrian origin has been derived by Price and Walker<sup>3,5</sup>. It has been applied with success by Mehta and Nagpaul<sup>6</sup>.

The density  $\rho_s$  of fossil fission tracks due to spontaneous fission of  $U^{238}$  present on any cleaved surface is given by

$$\rho_s = \frac{n_{238} \lambda_f R_0 (e^{\lambda_d T} - 1)}{2 \lambda_d} \quad (1)$$

where  $n_{238}$  is the number of  $U^{238}$  atoms/c.c. of the mineral,  $\lambda_f$  and  $\lambda_d$  are constants for spontaneous fission and  $\alpha$ -decay respectively,  $R_0$  is the combined range of fission fragments in mineral and  $T$  is the time since the sample started registering the tracks after it was crystallised.

The concentration of  $U^{238}$  in the sample is determined by measuring the concentration of  $U^{235}$ , which is done by irradiating the sample with a known dose of thermal neutrons in a nuclear reactor and measuring the density  $\rho_i$  of induced fission tracks on a newly cleaved surface.

$$\rho_i = n_{235} \sigma \phi R_0 / 2 \quad (2)$$

where  $n_{235}$  is number of  $U^{235}$  atoms/c.c. of mineral,  $\sigma$  is cross section for thermal neutron capture and  $\phi$  is the integrated (nvt) thermal neutron dose.

Eliminating  $R_0$  from (1) and (2),

$$T = \frac{1}{\lambda_d} \log_e \left( 1 + \frac{\rho_s I \phi \sigma \lambda_d}{\rho_i \lambda_f} \right) \quad (3)$$

where  $I$  is isotopic abundance of  $U^{235}$ . The values of various constants of eqn. (3) are:

$$\lambda_f = 7.03 \times 10^{-17} \text{ yr}^{-1}$$

$$\sigma = 582 \times 10^{-24} \text{ cm}^2$$

$$I = 7.26 \times 10^{-3}$$

$$\lambda_d = 1.52 \times 10^{-10} \text{ yr}^{-1}$$

Substituting these values, the eqn. (3) reduces to age formula

$$T = 6.57 \times 10^9 \log_e \left( 1 + 9.25 \times 10^{-18} \frac{\rho_s}{\rho_i} \phi \right) \quad (4)$$

**Experimental Details.**—The muscovite samples selected for this study have been collected from the mica mines of Kodarma, District Hazaribag (Bihar). The tight books of muscovite were chosen, washed and cleaved along the horizontal plane to expose fresh surfaces for etching of radiation damage. The samples were cut to the dimensions 2 cm.  $\times$  1.5 cm.  $\times$  200  $\mu$ m.

The samples are etched by immersing them for 1 hour in 48% HF at room temperature. The radiation damaged portions are attacked by chemical action and after washing and drying of samples the fossil fission tracks can be observed under the optical microscope using a magnification of 300–600 $\times$ . The tracks appear as dark black rods and can be easily counted to find the surface density  $\rho_s$ . Care should be taken to count the tracks only on the cleaved surface without going inside the sample.

To determine the induced fission track density  $\rho_i$ , the samples are prepared from the original tight books of muscovite, washed with de-ionised water and alcohol respectively and packed in lexan bags to fit a cylinder of diameter 1.6 cm and of length 3.5 cm. These samples are sent to the Isotope Division, B.A.R.C., Trombay, for irradiation with an integral neutron dose of  $10^{18}$  which is determined by enclosing a calibrated glass dosimeter along with the samples.

The induced fission of  $U^{235}$  takes place and the fission fragment tracks are deposited inside the samples as well as the glass dosimeter. The samples are etched as previously and the surface

density  $\rho_i$  of induced fission tracks is determined. The glass piece is fractured to expose the fresh surface and etched in 20% HF for 30 seconds at room temperature. The surface density  $\rho$  of etch pits is measured and the integral neutron flux  $\phi$  is calculated by using the calibration relation<sup>7</sup>,

$$\phi = 2.26 \times 10^{11} \rho \quad (5)$$

**Results.**—From the measured values of  $\rho_s$ ,  $\rho_i$  and  $\phi$ , the fission track ages of muscovite samples have been calculated from relation (4). The results are summarized in Table I. It is evident that the

TABLE I  
Fission track ages of Bihar Mica Belt (Kodarma Muscovites)

| Total neutron dose (nvt) = $4.3 \times 10^{18}$  |                              |                              |                           |
|--|------------------------------|------------------------------|---------------------------|
| Radiometric age <sup>8-10</sup> (m.Y) = 840–1100 |                              |                              |                           |
| Sample No.                                       | $\rho_s$ (cm <sup>-2</sup> ) | $\rho_i$ (cm <sup>-2</sup> ) | T Fission track age (m.Y) |
| 1  | 20                           | 5415                         | 901 ± 212                 |
| 2  | 19                           | 5531                         | 844 ± 194                 |
| 3  | 20                           | 5708                         | 856 ± 196                 |
| 4  | 23                           | 5354                         | 1011 ± 206                |
| 5  | 19                           | 5336                         | 865 ± 199                 |
| 6  | 19                           | 5460                         | 850 ± 195                 |
| 7  | 16                           | 5796                         | 679 ± 176                 |
| 8  | 19                           | 5681                         | 821 ± 188                 |
| 9  | 20                           | 5045                         | 957 ± 220                 |
| 10   | 20                           | 5768                         | 844 ± 194                 |
|  |                              |                              | Mea. 865 ± 72             |

ages lie between 700–1100 m.y. The average age comes out to be  $865 \pm 72$  million year which agrees with other age determinations made by radiometric methods<sup>8-10</sup>.

#### ACKNOWLEDGEMENTS

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## THERMAL DECOMPOSITION KINETICS

### Part VI\*. An Absolute-Rate-Theory-Based Equation for the Evaluation of Kinetic Parameters from Nonisothermal Thermogravimetry

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#### ABSTRACT

A new equation, taking into account the temperature-dependence of the pre-exponential factor based on the absolute rate theory of reactions, is proposed for the evaluation of kinetic parameters of solid-state reactions from nonisothermal thermogravimetric data.

THE rate equation widely used by different workers in nonisothermal thermogravimetry (TG) can be put in the form<sup>6</sup>

$$da/dT = (A/\phi) (1 - a)^n \exp(-E/RT) \quad (1)$$

where  $a$  = fraction decomposed at temperature  $T$ ,  $\phi$  = heating rate,  $A$  = pre-exponential term,  $R$  = gas

constant and  $E$  = energy of activation. In integrating eq. (1) it has always been assumed that  $A$  is temperature-independent<sup>7-11</sup>. Ingraham and Marier point out that this assumption is not rigorously valid<sup>12</sup>. A critical review of this aspect has recently been made by Gyulai and Greenhow<sup>13</sup>. In this communication, we wish to present the derivation of a more accurate equation taking into account the temperature-dependence of  $A$ .

\* For Parts I to V, see references 1 to 5.

## DERIVATION

According to the theory of absolute reaction rates, the temperature-dependence of  $A$  is given by the relation,

$$A = (kT/h) \exp(\Delta S/R) \quad (2)$$

where  $k$  = Boltzmann constant,  $h$  = Planck constant,  $\Delta S$  = entropy of activation. Combining eqs. (1) and (2), we get,

$$da/dT = [(k/h\phi) \exp(\Delta S/R)] \times \left[ T \exp\left(-\frac{E}{RT}\right) \right] (1-a)^n \quad (3)$$

Eq. (3) will be most useful in its integrated form. The integration may be done as follows:

Substituting

$$y = (k/h\phi) \exp\left(\frac{\Delta S}{R}\right)$$

and

$$x = E/RT$$

in eq. (3), rearranging it and putting in the integral form (limits for  $a$  and  $T$  being 0 to  $a$  and 0 to  $T$  respectively), we get

$$\int_0^a da/(1-a)^n = (YE^2/R^2) \int_x^\infty x^{-3} e^{-x} dx \quad (4)$$

The R.H.S. of eq. (4) is a special case of the most general form of the incomplete gamma function<sup>14</sup>. Eq. (4) on integration gives

$$\frac{1 - (1-a)^{1-n}}{1-n} = \frac{YE^2}{R^2} e^{-x} x^{-3} \left(1 - \frac{3}{x}\right) \quad (5)$$

Here the series term in R.H.S. has been terminated with the second term. Putting the L.H.S. as  $g(a)$  and re-introducing the values of  $x$  and  $Y$  eq. (5)

$$g(a) = (k/h\phi) \exp(\Delta S/R) \frac{RT^3}{E} \left(1 - \frac{3RT}{E}\right) \times \exp\left(-\frac{E}{RT}\right) \quad (6)$$

Taking logarithms,

$$\log\left(\frac{g(a)}{T^3}\right) = \log\left[\frac{kR}{h\phi E} \left(1 - \frac{3RT}{E}\right)\right] + \frac{\Delta S}{2 \cdot 303R} - \frac{E}{2 \cdot 303RT} \quad (7)$$

Graphical Evaluation of  $E$  and  $\Delta S$  from Eq. (7)

Two approaches are possible here:

(a) As in the Coats-Redfern equation, where  $2RT/E$  is assumed to be negligible compared to unity, one may assume that the same holds good for the term  $3RT/E$ . Then, eq. (7) reduces to:

$$\log\left[\frac{g(a)}{T^3}\right] = \log\left(\frac{kR}{h\phi E}\right) + \frac{\Delta S}{2 \cdot 303R} - \frac{E}{2 \cdot 303RT} \quad (8)$$

A plot of L.H.S. versus  $1/T$  would now be linear, with slope equal to  $-E/2 \cdot 303R$ . The value of  $\Delta S$  may be got from the intercept.

(b) The R.H.S. of eq. (7) may be rearranged in the form

$$\log\left(\frac{kR}{h\phi E}\right) + \frac{\Delta S}{2 \cdot 303R} + \left[\log\left(1 - \frac{3RT}{E}\right) - \frac{E}{2 \cdot 303RT}\right] \quad (9)$$

where the terms involving variable  $T$  have been segregated into the expression within the square bracket. This expression can be put in the form (here  $x = E/RT$ ):

$$\left[\log\left(1 - \frac{3}{x}\right) - \frac{x}{2 \cdot 303}\right] \quad (10)$$

This may be linearised by using the method of least squares, giving various values for  $x$  ranging<sup>15</sup> from 20 to 100. This gives

$$\log\left(1 - \frac{3}{x}\right) - \frac{x}{2 \cdot 303} = -0.0629 - 0.4337x \quad (11)$$

Resubstituting for  $x$ , we may now rewrite eq. (7) as

$$\log\frac{g(a)}{T^3} = \log\frac{kR}{h\phi E} + \frac{\Delta S}{2 \cdot 303R} - 0.0629 - \frac{0.4337E}{RT} \quad (12)$$

A plot of L.H.S. versus  $1/T$  would now be linear, from whose slope and intercept,  $E$  and  $\Delta S$  respectively may be evaluated. A further mathematical simplification may, if desired, be effected by taking over the  $T^3$  term also to the R.H.S. of eq. (7) and linearising, to give

$$\log g(a) = \log\frac{kE^2}{h\phi R^3} + \frac{\Delta S}{2 \cdot 303R} - 3.7958 - \frac{0.4582E}{RT} \quad (13)$$

## Comparison with the Coats-Redfern Equation

It is interesting to compare eq. (7) with the equation developed earlier by Coats and Redfern<sup>7</sup>, who did not take into account the temperature-dependence of  $A$ . The Coats-Redfern equation can be put in the following form:

$$\log\left(\frac{g(a)}{T^2}\right) = \log\left[\frac{AR}{\phi E} \left(1 - \frac{2RT_g}{E}\right)\right] - \frac{E}{2 \cdot 303RT} \quad (14)$$

or

$$\log\left(\frac{g(a)}{T^2}\right) = \log\left[\frac{kRT_g}{h\phi E} \left(1 - \frac{2RT_g}{E}\right)\right] + \frac{\Delta S}{2 \cdot 303R} - \frac{E}{2 \cdot 303RT} \quad (15)$$

It may be seen that equations (15) and (7) are generally of the same form. But, eq. (7) has the following advantages over eq. (15).

1. Eq. (7) has a more rigorous treatment and takes account of the temperature-dependence of  $A$ . At the same time, it involves just the same type of computational work as eq. (15).



2. Eq. (7) avoids the arbitrariness with which eq. (15) introduces the temperature term in the expression for

$$A = \frac{kT}{h} e^{\Delta S_{IR}}$$

the  $T$  in this expression has been variously and arbitrarily chosen as  $T_s$  (the DTG peak temperature)<sup>2</sup> or  $\bar{T}$  (the average temperature)<sup>16</sup>.

The improvement effected by the present equation may be illustrated by considering a theoretical TG curve. Such a procedure has been suggested by Sestak in a different context<sup>17</sup>. A theoretical TG curve with an assumed value of  $E = 120$  kJ mole<sup>-1</sup> was used for this purpose. Here, the Coats-Redfern equation gives a value  $E = 123.3$  kJ mole<sup>-1</sup> whereas the present equation gives a value  $E = 119.8$  kJ mole<sup>-1</sup>. The improvement is obvious.

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## TWO UNUSUAL FLAVONES (ARTEMETIN AND 7-DESMETHYL ARTEMETIN) FROM THE LEAVES OF *VITEX TRIFOLIA*

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#### ABSTRACT

Adsorption chromatography on silica gel of the chloroform extract of dry leaves of *Vitex trifolia* (Verbenaceae) has yielded two methylated flavones of rare occurrence. Based on chemical as well as UV, IR, PMR and Mass spectral data, the major compound has been characterised as 5, 7-dihydroxy-3, 3', 4', 6-tetra methoxy flavone (3, 3', 4', 6-tetra methyl quercetagenin) and the minor as artemetin (5-hydroxy-3, 3', 4', 6, 7-penta methoxy flavone) by direct comparison with authentic sample. The earlier observation regarding the variation of flavonoid pattern with reference to plant geography in *Vitex* is further supported by our results.

THE distribution of flavonoids in the genus *Vitex*<sup>1</sup> under the family Verbenaceae and the Natural Order Tubiflorae is interesting especially with reference to the glycoflavones (atypical in this family) and the unusual flavones like casticin (3, 4', 6, 7-tetra methyl quercetagenin), and certain variations in the flavonoid patterns relating to plant geography have been observed. Some significance is also attached to these flavonoids in the classification of plants of this family.

In continuation of our work on the flavone glucuronides of the Verbenaceae<sup>2</sup>, we have made a

detailed chemical examination of the flavonoids of the leaves of *Vitex trifolia* Linn. earlier recorded to contain casticin<sup>3</sup> and vitexin<sup>4</sup>, and our results are recorded here.

The dry leaves of *V. trifolia* were first extracted with hot  $\text{CHCl}_3$  and then with MeOH. The concentrated  $\text{CHCl}_3$  extract was chromatographed on a column of silica gel using petroleum ether, benzene and  $\text{CHCl}_3$  as eluting solvents. No crystalline flavonoid was obtained from petrol eluates.

The residue from the benzene fraction on recrystallisation from  $\text{Me}_2\text{CO-MeOH}$  yielded a small

amount of light yellow needles of a flavone, m.p. 162–63°,  $\lambda_{\max}$  254, 274 sh. 346 nm. (MeOH). It was purple under U.V. and U.V./NH<sub>3</sub>. It gave an olive green colour with Fe<sup>3+</sup> and yielded quercetagenin on demethylation with Ac<sub>2</sub>O and HI. It had  $R_f$ : 0.36 (15% HOAc), 0.78 (30% HOAc), 0.85 (50% HOAc), 0.95 (BAW), 0.92 (phenol), 0.96 (Forestral) and 0.96 (*t*-BAW). It was identified as 5-hydroxy-3, 3', 4', 6, 7-penta methoxy flavone (artemetin<sup>5,6</sup>) and the identity further confirmed by direct comparison including co-PC with an authentic sample. A minute quantity of a flavone present in the mother liquor was fluorescent light blue under U.V. and U.V./NH<sub>3</sub> and did not give any colour with Fe<sup>3+</sup>. It was identified as 5-methyl artemetin (quercetagenin hexa methyl ether) by direct comparison with a synthetic sample prepared by complete methylation of quercetagenin.

The residue from the CHCl<sub>3</sub> fraction was thrice recrystallised from Me<sub>2</sub>CO–MeOH, when a pale yellow flavone, m.p. 168–69° was obtained. It gave yellow colour with NH<sub>3</sub> and greenish blue with Fe<sup>3+</sup>. It was deep purple under U.V. and U.V./NH<sub>3</sub> and had  $\lambda_{\max}$ : 256, 271 sh. 340 (MeOH); 256, 271, 344 (NaOAc); 266, 282 sh. 297 sh. 368 (AlCl<sub>3</sub>) and 257, 272, 342 (NaOAc/H<sub>3</sub>BO<sub>3</sub>) almost the same as artemetin. The PMR spectrum (CDCl<sub>3</sub>) showed signals ( $\delta$  values, ppm) at 3.98, 3.95, 3.90 and 3.88 (each singlet of 3 protons due to –OCH<sub>3</sub>), 6.5 (singlet, 8–H), 6.63 (doublet,  $J = 9$  cps, 5–H) 7.66 (multiplet, 2–H and 6'–H) and a low field proton at 12.3 (5–OH) and the IR (KBr) exhibited absorption bands (cm<sup>-1</sup>) at 3640 (–OH), 1670 (conjugated C=O), 1610, 1590, 1560 and 1520 (benzene derivative). The mass spectrum of the compound showed the parent ion at  $m/e$  374 ( $M^+$ , C<sub>19</sub>H<sub>18</sub>O<sub>8</sub>, 100%) and fragmentation ions at 373 ( $M^+-H$ , 24%), 359 ( $M^+-CH_3$ , 33%), 346 ( $M^+-CO$ , 2%), 344 ( $M^+-2CH_3$ , 5%), 331 ( $M^+-CH_3CO$ , 10%), 187 ( $M^+$ , 20%) and 173 ( $M^+-2H$ , 35%) and 182, 167, 161, 151, 139, 137 and 123 (due to ions of RDA fragmentation and further cleavage). The PMR and mass spectral data along with the  $R_f$  (0.20, 0.56, 0.79, 0.92, 0.92, 0.93 and 0.94 resp. in the above solvents) clearly indicated the compound to be a dihydroxy-tetra methoxy flavone. On acetylation with Ac<sub>2</sub>O and pyridine, it yielded a crystalline diacetate, m.p. 174–75°, whose mass spectrum showed the parent ion at  $m/e$  458 ( $M^+$ , C<sub>23</sub>H<sub>22</sub>O<sub>10</sub>, 11.5%) and fragmentation ions at 457 ( $M^+-H$ , 14%), 443 ( $M^+-CH_3$ ), 428 ( $M^+-2CH_3$ ), 415 ( $M^+-CH_3CO$ , 100%), 400 [ $M^+-(CH_3CO+CH_3)$ , 23%], 385 [ $M^+-(2CH_3+CH_3CO)$ ] 372 ( $M^+-2CH_3CO$ , 35%) and 342 [ $M^+-(2CH_3+2CH_3CO)$ ] confirming the dihydroxy tetra methoxy flavone structure,

On demethylation with HI and Ac<sub>2</sub>O, it gave 3, 3', 4', 5, 6, 7 hexahydroxy flavone (quercetagenin). From these data, the flavone was identified as a tetra methyl ether of quercetagenin. The almost identical UV spectra of the compound and artemetin, and the mass fragmentation pattern established the structure as 5, 7-dihydroxy-3, 3', 4', 6-tetra methoxy flavone. (The 7-OH of a 6-methoxylated flavone has been recorded<sup>7,8</sup> to behave in such a manner as to miss its detection by U.V. analysis). The identity was finally confirmed by a partial synthesis of artemetin from our sample by selective methylation using Me<sub>2</sub>SO<sub>4</sub> and anhydrous K<sub>2</sub>CO<sub>3</sub> for 6 hr. (Selective methylation using Me<sub>2</sub>SO<sub>4</sub> and KHCO<sub>3</sub> for 12 hr was not successful, which may again be attributed to the peculiar nature of 7-OH in 6-methoxylated flavones).

The pigment from MeOH concentrate was identified as luteolin by  $\lambda_{\max}$ ,  $R_f$ , preparation of its tetraacetate and direct comparison with an authentic sample. No flavone glycoside could be detected.

Our isolation of artemetin and 5, 7-dihydroxy-3, 3', 4', 6-tetramethoxy flavone (7-desmethyl artemetin) in the place of casticin and vitexin reported earlier in *V. trifolia* confirms the observation of Harborne<sup>1</sup> of the variation of flavonoid pattern in relation to plant geography in this genus. Artemetin has been earlier isolated from the leaves<sup>9</sup> and seeds<sup>10</sup> of *V. negundo*. Ours is the second report of the isolation of the unusual flavone, 7-desmethyl artemetin, the first being from *Bahia oppositifolia*<sup>11</sup> (Compositae).

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## AEROPALYNOLOGICAL STUDIES OF BANGALORE CITY

### Part I. Pollen Morphology of *Parthenium hysterophorus* Linn.

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**B**ANGALORE CITY, known for its salubrious climate almost throughout the year, is often referred to as the air-conditioned City of India. However, the atmosphere of the City is full of pollen pollutants. This fact has great bearing on the different types of pollen allergies, much prevalent in this City.

A comprehensive research scheme has been undertaken by the authors to tackle the pollen allergy problem from palynological point of view. As a prerequisite to this research project, construction of pollen flora based on the collection of pollen from plants growing in the City has been undertaken.

Of late several reports have been published on allergic manifestations of the recently introduced notorious weed, commonly referred to as Congress Weed or White Top, and botanically known as *Parthenium hysterophorus* Linn. It has been further reported that the food grains imported into India from U.S.A. and Canada were contaminated with the seeds of this weed<sup>1,2</sup>. Several methods of eradication of this fast spreading weed, have been suggested recently by Vartak<sup>3</sup> and Jayachandra<sup>4</sup>.

*Parthenium hysterophorus* Linn., a member of the family Compositae, is known to produce pollen abundantly. The toxic effects of the pollen grains of this weed with reference to allergies have been reported by Wodehouse<sup>11</sup>, Shivpuri *et al.*<sup>5,7</sup>. The flowers of this weed known to be amphiphilous. The prevalence of these pollen grains in the atmosphere has been reported by the Aeropalynological work carried on by Shivpuri *et al.*<sup>7</sup> at Delhi and the same has been confirmed by us at Bangalore.

The pollen grains of *P. hysterophorus* were studied by using the standard palynological techniques of Erdtman<sup>2,3</sup> and were found to be very interesting morphologically. Literature indicated that detailed pollen morphology of this weed has not been worked out. Hence the diagnosis of the pollen grains of this weed has been presented here.

### Pollen diagnosis of *P. hysterophorus* Linn.

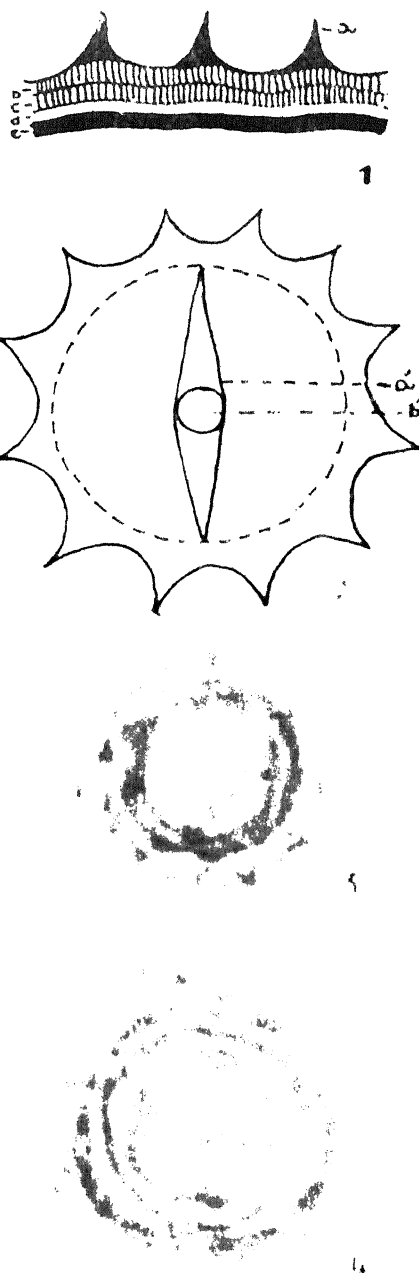
(Figs. 1-4)

Pollen grains 3-colporate (poritreme), oblatespheroidal ( $16 \times 17 \mu$ ). Apocolpium diameter about  $3.5 \mu$ . Colpi ( $10 \times 2 \mu$ ) tenuimarginate, with tapering ends, membrane smooth.

Ora circular (diameter about  $2 \mu$ ). Exine (spinules included) about  $4.4 \mu$  thick. Sexine about  $3 \mu$  thick, pertectate supratrigillate, provided with pointed spinules. Tegillum undulating, differentiated into supra and infrategillar layers, each less than  $0.5 \mu$  high, supporting the tegillum of each layer. Spinules about  $2 \mu$  high with pointed solid apices, base about  $2.2 \mu$  wide made up of slender rod like elements. Nexine as it seems, consists of a homogeneous layer, inner margin smooth. There appears to be a thin distinct region about less than  $0.5 \mu$  wide between the baculate layer and the nexine.

**Discussion and Summary.**—The family Compositae is referred to as a Eurypalynous family because of the great variety of pollen types found in its members. As far as pollen morphology of *P. hysterophorus* is concerned, except Wodehouse's<sup>10</sup> casual reference, no detailed description is available in the literature surveyed so far. Taking into consideration the pollen characters, Wodehouse's<sup>10,11</sup> supports the view expressed by Bentham and Hooker (1873) who state that phylogenetically Ambrosieae (ragweed tribe) shows a close relationship with the tribe Helianthae through Melampodinae, a sub tribe of Helianthae including *Parthenium* and *Parthenice*.

Considering the views expressed by Wodehouse and our observations of the pollen morphology of *P. hysterophorus* in which the grains are typically 3-colporate, oblatespheroidal, spinulose, a characteristic feature of the majority of the members of Helianthae (Sunflower tribe) and the Ambrosieae, it can be concluded that the tribes Helianthae and



FIGS 1-4. Pollen grains of *Parthenium hysterophorus* Linn. Fig. 1. Diagrammatic representation of the exine stratification, (a) Spindle, (b) supporting bacula of the suprategillum, (c) supporting bacula of infrategillum, (d) thin region inbetween the baculate layer and the nexine, (e) nexine. Fig. 2. Diagrammatic representation of the pollen

granum in apical view showing the apertures, aperture in apical view. Fig. 3. Photomicrograph of the equatorial view showing the aperture and the apertures. Fig. 4. Photomicrograph of the grain in apical view.

Amforaceae are closely related through Melanopodaceae including *Parthenium* and *Parthenium*.

Similarly, the presence of a thin distinct region inbetween the nexine and the baculate layer as shown by Raj in the pollen grains of *Helianthus annuus* Linn. is also noticed by us in the pollen grains of *P. hysterophorus*. Wodehouse<sup>10</sup> while comparing the grains of *Parthenium molle* and *Parthenium* states that they are trigonate with long furrows reaching almost from pole to pole. However, in the illustration of *Parthenium molle* given by him, a clear corporate condition is seen.

The present investigation has brought forth a clear idea of the pollen morphology of the weed *P. hysterophorus* which according to Shrivastava and Kartar Singh<sup>7</sup> is known to cause skin allergies. However, further work is needed to find out the representation of pollen grains of *P. hysterophorus* in the atmosphere and the different types of allergies caused by them. It is planned to undertake the abovementioned work in the second phase of our research project.

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## LETTERS TO THE EDITOR

## INFRARED AND ULTRAVIOLET SPECTRA OF DI-SUBSTITUTED TOLUENES (5, 2-, 6, 3- AND 2, 4- CHLORO-FLUOROTOLUENES)

ALTHOUGH the infrared and ultraviolet absorption spectra of toluenes, mono substituted toluenes<sup>1</sup> and di-substituted toluenes<sup>2-6</sup> were investigated, fluorinated di-substituted toluenes still require a systematic study. With this in view the study of the spectra of the above-mentioned molecules has been carried out. The present communication reports the results of preliminary investigations of infrared spectra of 5, 2- and 2, 4-Chloro-fluorotoluenes and ultra-violet spectra of 5, 2- and 6, 3-Chloro-fluorotoluenes (hereafter referred to as 5, 2-CFT, 2, 4-CFT and 5, 3-CFT).

The infrared spectra were obtained on a Perkin Elmer 521 grating spectrophotometer. The ultra-violet absorption spectra were photographed with cell lengths of 50 and 70 cm on a Hilger medium quartz spectrograph. The temperature of the absorbing vapour was varied from 5° to 60° C. The time of exposure varied from 10 to 90 minutes. The chemicals were obtained from M/s. Fluka, AG, Switzerland.

All the three molecules belong to the  $C_s$  point group. The 39 fundamental vibrations of each molecule will divide into 27  $a'$  and 12  $a''$  vibrations. The transitions involved in the spectrum of each molecule would then be  $A' - A'$ , which is allowed and corresponds to  $B_{2u} + A_{1g}$  of benzene. Consequently the most intense band would be the (0, 0) band.

The most intense band at  $36136\text{ cm}^{-1}$  ( $2766.5\text{ \AA}$ ) in 5, 2-CFT and at  $36326\text{ cm}^{-1}$  ( $2752.0\text{ \AA}$ ) in 6, 3-CFT lying in the longer wavelength region of the spectra and which are observed even at lower temperatures, have been taken as the (0, 0) bands of the respective systems. In the absence of the Raman data and lack of details of the infrared band contours, the present assignments are tentative. However, a comparison with the data of related molecules shows that the assignments are in all probability correct. In order to support the vibrational assignments of 2, 5-CFT and 4, 2-CFT the corresponding frequencies of 2, 5-Di-chlorotoluene, as reported by Mehrotra<sup>3</sup> are mentioned in Table I.

The ultra-violet spectra of 5, 2-CFT and 6, 3-CFT have been analysed in terms of the following ground and excited states fundamental frequencies.

TABLE I

Infrared frequencies and their assignments

| 2, 5-CFT<br>Frequencies<br>$\text{cm}^{-1}$ |    | 4, 2-CFT<br>Frequencies<br>$\text{cm}^{-1}$ |    | 2, 5-Di-<br>chloro-<br>toluene | Assignments                       |
|---|----|---|----|--------------------------------|-----------------------------------|
| 365   | w  | 335   | m  | 404                            | $\delta$ (C—C—C)                  |
| 410   | w  | 410   | m  | 438                            | $\pi$ (C—C—C)                     |
| 435   | s  | 440   | m  |                                | $\beta$ (C—F)                     |
| 445   | tn | 457   | s  |                                | $\pi$ (C—C—C)                     |
| 545   | m  |   |    |                                | $\beta$ (C—CH <sub>3</sub> )      |
| 551   | w  | 565   | s  | 544                            | $\delta$ (C—C—C)                  |
| 665   | w  | 665   | w  | 700                            | $\gamma$ (C—Cl)                   |
| 700   | w  | 685   | s  |                                | $\pi$ (C—C—C)                     |
| 755   | s  | 740   | s  |                                | $\gamma$ (C—C)                    |
| 810   | vs | 800   | s  | 780                            | $\pi$ (C—H)                       |
| 870   | s  | 850   | s  | 884                            | $\pi$ (C—H)                       |
| 885   | s  | 900   | s  | 948                            | $\pi$ (C—H)                       |
| 995   | w  | 995   | m  | 1000                           | $\delta$ (C—C—C)                  |
| 1035  | w  | 1035  | s  | 1014                           | (C—H <sub>3</sub> )<br>Rocking    |
| 1085  | s  |   |    | 1064                           | $\beta$ (C—H)                     |
| 1175  | vs | 1175  | s  | 1140                           | $\beta$ (C—H)                     |
| 1230  | vs | 1230  | s  | 1268                           | $\gamma$ (C—CH <sub>3</sub> )     |
| 1235  | vs | 1230  | s  |                                | $\beta$ (C—H)                     |
| 1245  | m  | 1260  | s  |                                | $\gamma$ (C—F)                    |
| 1255  | m  | 1280  | m  | 1280                           | $\gamma$ (C—C)                    |
| 1375  | w  | 1375  | m  | 1396                           | $\delta$ (C—H <sub>3</sub> ) Sym  |
| 1395  | s  | 1390  | w  |                                | $\gamma$ (C—C)                    |
| 1440  | w  | 1430  | w  |                                | $\delta$ (C—H <sub>3</sub> ) Asym |
| 1485  | vs | 1485  | vs | 1480                           | $\gamma$ (C—C)                    |
| 1570  | w  | 1575  | s  | 1564                           | $\gamma$ (C—C)                    |
| 1595  | w  | 1595  | s  | 1592                           | $\gamma$ (C—C)                    |
| 2850  | w  | 2860  | w  | 2856                           | $\gamma$ (C—H <sub>3</sub> ) Sym  |
| 2920  | w  | 2920  | w  |                                |                                   |
| 2960  | w  | 2950  | w  | 2956                           | $\gamma$ (C—H <sub>3</sub> ) Asym |
| 2970  | w  | 2970  | w  | 2988                           |                                   |
| 3030  | w  | 3030  | w  |                                |                                   |
| 3080  | w  | 3080  |    | 3072                           | $\gamma$ (C—H)                    |

Notations :  $\gamma$ =Stretching,  $\delta$ =In plane ring deformation,  $\pi$ =Out of plane bending,  $\beta$ =Plane bending, w=Weak, m=Medium, s=Strong, vs=Very strong.

5, 2-CFT Ground state fundamentals—111, 208 and  $235\text{ cm}^{-1}$ ; Excited state fundamentals—608, 668, 724, 805, 986, 1070, 1109 and  $1316\text{ cm}^{-1}$ ; 6, 3-CFT Ground state fundamentals—206 and  $264\text{ cm}^{-1}$ ; Excited state fundamentals—398, 494, 585, 689, 1025, 1060 and  $1257\text{ cm}^{-1}$ .

The work on the infrared spectra of 6, 3-CFT is in progress. The study of Raman spectra of these molecules is also planned.

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### Rb-Sr RADIOMETRIC STUDIES FOR THE DALHOUSIE AND ROHTANG AREAS, HIMACHAL PRADESH

ONLY scanty work has been done so far, in the field of isotopic and geochronological studies for the Himalayas, by use of Rb-Sr technique. As an example, whole-rock Rb-Sr data are available<sup>1,3</sup> for only five samples from the vast Himalayan terrain in comparison to thousands of samples from the Alps. This note describes a part of the reconnaissance type of Rb-Sr whole-rock dating which has been undertaken at Chandigarh for the granitic and gneissic rocks of N-W Himalayas.

The geochemical procedures which are being used are similar to those described by Jager<sup>3</sup>. The equipment includes a Nier-type of mass spectrometer with 90-degree 25-cm-radius magnetic analyser, equipped with a thermal ionisation source, which was fabricated and provided to us at Chandigarh by the Bhabha Atomic Research Centre, Bombay. Rb<sup>87</sup> and Sr<sup>86</sup> spikes, obtained from the Oak Ridge Laboratory of U.S. Atomic Energy Commission, have been employed.

This note describes the analyses of two whole-rock samples and one biotite sample. Results are given in Table I.

Sample PHS/22 is well enriched in radiogenic strontium. The isochron-age is, therefore, estimated to be only slightly lower than the apparent age. The granitic sample PHS/17 from the same outcrop, but from a different location, gave an apparent age<sup>2</sup> of  $456 \pm 50$  million years. From the analyses of these two samples, one can tentatively conclude that the Dalhousie granitic outcrop consists of rocks of at least two ages of emplacement, viz., about  $350 \pm 50$  m.y. and about  $450 \pm 50$  m.y. One can further conclude that the minimum age of sedimentation of the surrounding rocks into which the Dalhousie granite appears to be intruding is about  $450 \pm 50$  m.y.

Results for the biotite clearly show the re-setting of the clock during Himalayan metamorphism, which indicates that the temperature rose to more than 300°C during this period. The true age of metamorphism, however, is expected to be quite a bit lower than the apparent age of the biotite, since the initial value of Sr<sup>87</sup>/Sr<sup>86</sup> after rehomogenisation of strontium during the tertiary period is expected to be much larger than the value of 0.709, that was assumed for calculating apparent ages. It will be useful to determine the true age of biotites in this region by use of whole-rock-biotite isochrons, as has been done for the Mandi granite<sup>1</sup>.

The sample MR/7 was collected on the south-side, but almost near the top of Rohtang pass. Since it is not well enriched in radiogenic strontium, its isochron age might turn out to be considerably smaller than the apparent age. The possibility that its true age is same as that of the Mandi granite cannot be ruled out.

As pointed out earlier<sup>2</sup>, the K-Ar mineral ages for this polymetamorphic region can provide a misleading picture, unless interpreted very carefully and cautiously. A comparison between Rb-Sr data and K-Ar data for this region is not considered fruitful at present, since the published data is rather sparse and inadequate for this comparison.

TABLE I

| Sample No. | Rock-type                         | Location (Longitude/Latitude)          | Rb <sup>87</sup> in ppm | Common Sr in ppm | Sr <sup>87</sup> /Sr <sup>86</sup> | Apparent age   |
|------------|-----------------------------------|--|-------------------------|------------------|------------------------------------|----------------|
| PHS/22     | Pegmatite                         | (76° 0'; 32° 32') near Dalhousie town) | 145.5                   | 16.0             | 1.191 ± 0.03                       | 350 ± 50 m.y.  |
| MR/7       | Gneiss                            | (77° 10'; 32° 17') near Rohtang pass   | 61.8                    | 62.0             | 0.80 ± 0.02                        | 612 ± 100 m.y. |
| ID/3       | Biotite, separated from a granite | (76° 01'; 32° 33') near Kala Topc      | 850                     | 14.6             | 1.24 ± 0.03                        | 61 ± 10 m.y.   |

Constants used; Decay constant =  $1.47 \times 10^{-11}$ /year; Rb<sup>86</sup>/Rb<sup>87</sup> = 2.591, Sr<sup>88</sup>/Sr<sup>86</sup> = 8.375 and (Sr<sup>37</sup>/Sr<sup>86</sup>)<sub>i</sub> = 0.709.

showed that solutions of  $\text{CuL}_2$  in  $\text{CCl}_4$  are most susceptible to photolysis and those in benzene least susceptible, (the other usual extractants falling between these two limits), these two solvents were chosen for a detailed study.

#### Studies in Diffused Day-light

Known volumes of these solutions were taken in clear-glass stoppered bottles and were exposed to diffused day-light for different periods. Measured aliquots were removed at definite time intervals and their absorbance was determined at 435 nm. ( $\text{CuL}_2$  has a characteristic absorption peak at 435 nm due to a ligand-to-metal charge transfer band.) It was found that  $\text{CCl}_4$  solutions deteriorated fast, whereas benzene solutions appeared to be much stabler. For instance, after a 4 hour exposure to diffused day-light, the absorbance of a typical solution fell from an initial value of 0.490 to 0.340 in  $\text{CCl}_4$  medium, while in a benzene medium, it dropped from 0.490 only to 0.475.

#### Studies Using a Mercury Arc Lamp

A low pressure mercury arc lamp (Hanovia, 4 Watts, 220 volts) and a water-cooled quartz photochemical cell with an inner thimble (capacity 1 litre) were used. Standard  $\text{CuL}_2$  solutions were irradiated at two temperatures,  $30^\circ \pm 0.1$  and  $36^\circ \pm 0.1$ . The results indicated that temperature variations, as above, have practically no effect on the decomposition rate. The results of a typical study at  $30^\circ$  are given in Table I.

TABLE I  
Photochemical decomposition of  $\text{CuL}_2$  using  
4 Watts UV lamp

| Time<br>hours | Absorbance in<br>$\text{CCl}_4$ | Absorbance in<br>benzene |
|---------------|---------------------------------|--------------------------|
| 0             | 2.060                           | 2.120                    |
| 0.5           | 1.960                           | 2.090                    |
| 1.0           | 1.855                           | 2.070                    |
| 1.5           | 1.700                           | 2.040                    |
| 2             | 1.501                           | 2.010                    |
| 3             | 1.344                           | 2.000                    |
| 4             | 1.074                           | 1.990                    |
| 5             | 1.038                           | 1.970                    |
| 6             | 0.859                           | 1.960                    |
| 8             | 0.830                           | 1.910                    |
| 12            | 0.650                           | 1.840                    |
| 16            | 0.286                           | 1.820                    |
| 20            | 0.120                           | 1.770                    |
| 24            | 0.096                           | 1.665                    |

$\text{CCl}_4$ :  $k = 3.57 \times 10^{-5} \text{ sec}^{-1}$ ;  $\phi = 0.081$ ;  $t_{1/2} = 5.4$  hours. Benzene:  $k = 2.99 \times 10^{-6} \text{ sec}^{-1}$ ;  $\phi = 0.0073$ ;  $t_{1/2} = 64.4$  hours.

The progress of the substrate disappearance was followed by withdrawing small measured aliquots (2 ml) at definite time intervals, diluting to 10 ml and determining the absorbance values ( $A$ ) at 435 nm. Since the volume of the aliquot removed (2 ml) is very small in comparison with the volume of the system (1000 ml), the volume effect is ignored in the present study. A plot of  $\log (A_0/A_t)$  against time ( $t$ ) was found to be a straight line both in  $\text{CCl}_4$  and in benzene, where  $A_0$  and  $A_t$  denote absorbance at  $t = 0$  and  $t = t$ . This implies conformity with the first order kinetics. The rate constant for substrate disappearance ( $k$ ) was found to be  $3.57 \times 10^{-5} \text{ sec}^{-1}$  in  $\text{CCl}_4$  and  $2.99 \times 10^{-6} \text{ sec}^{-1}$  in benzene. The values for the time of half change are:  $t_{1/2} = 5.4 \text{ hr}$  in  $\text{CCl}_4$  and  $t_{1/2} = 64.4 \text{ hr}$  in benzene. An increase of temperature from  $30^\circ$  to  $36^\circ$  did not significantly affect the value of  $k$ . A preliminary determination of the average quantum efficiency of the decomposition gave values of  $\phi = 0.081$  in  $\text{CCl}_4$  and  $\phi = 0.0073$  in benzene.

It was observed that a solid residue was thrown down in the  $\text{CCl}_4$  medium during the course of the decomposition. This is analysed for  $\text{Cu}^{2+}$ ,  $\text{Cl}^-$  and elemental sulphur. The decomposition reaction thus appears to be complicated, as is the case with several photolytic reactions of co-ordination complexes<sup>1</sup>.

From these studies, it is clear that benzene is a better solvent than  $\text{CCl}_4$  for the spectrophotometric determination of copper.

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#### MESOMORPHIC PROPERTIES OF SOME BIPHENYL BENZOATES

SEVERAL biphenyl esters exhibit mesomorphic properties<sup>1,2</sup>. As a part of our programme of study of the structure and properties of liquid crystals, we have synthesized a homologous series of ten biphenyl-4-*p-n*-alkoxybenzoates starting from *p-n*-

alkoxybenzoic acids through their acid chlorides. The transition temperatures and elemental analyses are given in Tables I and II respectively.

TABLE I

Transition temperatures of biphenyl 4-*p*-*n*-alkoxy benzoates

| Compound number | R <sup>a</sup>                  | Transition temperatures °C | Δ T °C nematic range |
|-----------------|---------------------------------|----------------------------|----------------------|
| 1               | CH <sub>3</sub>                 | 157 -157.5 (145)           | ..                   |
| 2               | C <sub>2</sub> H <sub>5</sub>   | 161 -162 (157.5)           | ..                   |
| 3               | C <sub>3</sub> H <sub>7</sub>   | 146 -147 (136)             | ..                   |
| 4               | C <sub>4</sub> H <sub>9</sub>   | 158 -159 (142.5)           | ..                   |
| 5               | C <sub>5</sub> H <sub>11</sub>  | 144 -145 (113.5)           | ..                   |
| 6               | C <sub>6</sub> H <sub>13</sub>  | 132.5 -135.5               | 3                    |
| 7               | C <sub>7</sub> H <sub>15</sub>  | 128 -130                   | 2                    |
| 8               | C <sub>8</sub> H <sub>17</sub>  | 120 -131                   | 11                   |
| S               |                                 |                            |                      |
| 9               | C <sub>10</sub> H <sub>21</sub> | 111 -126.5 (106)           | 15.5                 |
| S               |                                 |                            |                      |
| 10              | C <sub>12</sub> H <sub>25</sub> | 110.2 = 113.2 - 124.5      | 11.3                 |

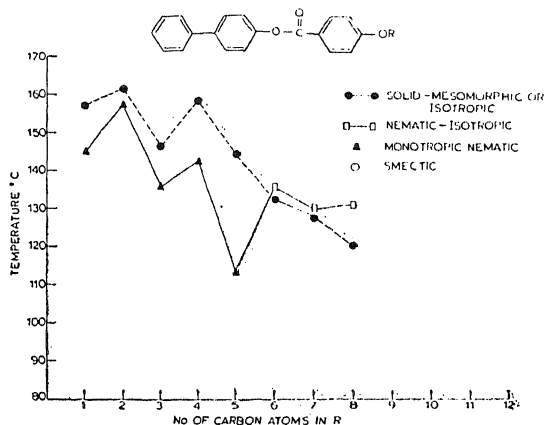
<sup>a</sup> All alkyl (R) groups are normal. Temperatures in parentheses indicate monotropic transitions. S indicates the occurrence of a smectic phase.

TABLE II

| Compound number | Molecular formula                              | Calculated % |       | Found % |       |
|-----------------|--|--------------|-------|---------|-------|
|                 |  | C            | H     | C       | H     |
| 1               | C <sub>20</sub> H <sub>16</sub> O <sub>3</sub> | 78.94        | 5.262 | 78.78   | 5.50  |
| 2               | C <sub>22</sub> H <sub>18</sub> O <sub>3</sub> | 79.25        | 5.661 | 79.52   | 5.763 |
| 3               | C <sub>24</sub> H <sub>20</sub> O <sub>3</sub> | 79.52        | 6.024 | 79.50   | 6.280 |
| 4               | C <sub>26</sub> H <sub>22</sub> O <sub>3</sub> | 79.77        | 6.357 | 79.64   | 6.45  |
| 5               | C <sub>28</sub> H <sub>24</sub> O <sub>3</sub> | 80.00        | 6.766 | 80.00   | 6.94  |
| 6               | C <sub>26</sub> H <sub>26</sub> O <sub>3</sub> | 80.21        | 6.952 | 80.19   | 7.18  |
| 7               | C <sub>26</sub> H <sub>28</sub> O <sub>3</sub> | 80.42        | 7.218 | 80.55   | 7.57  |
| 8               | C <sub>27</sub> H <sub>30</sub> O <sub>3</sub> | 80.60        | 7.463 | 80.22   | 7.63  |
| 9               | C <sub>29</sub> H <sub>32</sub> O <sub>3</sub> | 81.08        | 8.108 | 81.14   | 7.98  |
| 10              | C <sub>31</sub> H <sub>34</sub> O <sub>3</sub> | 81.21        | 8.296 | 81.32   | 8.28  |

The first five members of the series exhibit a monotropic nematic mesophase. The hexyl, heptyl, and octyl derivatives are enantiotropic nematic. The smectic mesophase appears at the decyl derivative as a monotropic phase. The plot of the transition temperatures *versus* the number of carbon atoms in the alkyl chain is given in Fig. 1, and shows the usual odd-even effect. The thermal stability of the nematic phase decreases with increasing carbon chain length. It is interesting to note that there is alternation in the crystal to isotropic transition temperatures of the first five members of the series. This alternation is attributed to a similarity in crystal structure, as evidenced from the X-ray study<sup>3</sup> of the higher homologues of *p*-*n*-alkoxybenzoic acids. These esters are thermally

less stable than the corresponding Schiff's bases reported by Gray *et al.*<sup>4</sup>



The transition temperatures were determined in open capillary tubes using a polarizing microscope (Franz Kustner Nacht KG, Dresden, Model HMK 70/3171) in conjunction with a heated stage. Infrared spectra were recorded (nujol mull) on a Perkin-Elmer spectrophotometer, Model 700 and NMR spectra were recorded on a Varian T-60 spectrometer, in CDCl<sub>3</sub> using tetramethylsilane as internal standard. The *p*-*n*-alkoxybenzoic acids were prepared according to the method of Lauer *et al.*<sup>5</sup> A typical procedure for the preparation of the ester is given below.

**Biphenyl-4-*p*-*n*-butoxy benzoate.**—*p*-*n*-Butoxybenzoic acid (3.88 g) was refluxed for 3 hours with thionyl chloride (12 ml) using a drop of pyridine. Excess of thionyl chloride was removed under reduced pressure. 4-Hydroxy biphenyl (3.4 g) in dry pyridine (60 ml) was added in one lot to the crude acid chloride, the mixture stirred for 3 hours at room temperature and left overnight. The reaction mixture was poured onto a stirred mixture of concentrated hydrochloric acid and crushed ice, when the ester precipitated out. It was filtered, washed with 10% sodium hydroxide solution, water and dried. Yield, 6.55 g.

The crude ester was chromatographed on neutral alumina (NCl, Poona, Brockmann activity 1) and was eluted with benzene. Removal of the solvent from the eluate afforded a white material, which was crystallized from benzene-light petroleum, m.p. 158–159° C.

$\nu_{\text{max}}$  1720, 1605, 1580, 1510, 1170, 980 and 760 cm<sup>-1</sup>.  $\delta$ , 1.0 (s, 3 H, -CH<sub>3</sub>); 1.2–2.0 (m, 4 H); 4.18 (t, 2 H, -OCH<sub>2</sub>); 7.0 (d, J=8 Hz, 2 H); 7.2, 7.6 (m, 9 H); 8.2 (d, J=8 Hz, 2 H).



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#### A NEW VOLUMETRIC METHOD FOR THE DETERMINATION OF COPPER

We have developed a simple and quick method of determining copper which is based on the reduction of cupric salts with stannous chloride, removal of the excess of the stannous chloride by reaction with mercuric chloride and then oxidising the cuprous salt back to cupric salt with ferric sulphate which results in the production of an equivalent quantity of ferrous sulphate which is then titrated against a standard solution of potassium dichromate. The method is thus very similar to the well-known stannous chloride reduction method of the determination of iron, the major difference being that an inert atmosphere has to be maintained during the time of the removal of the excess stannous chloride with mercuric chloride and till the addition of the ferric sulphate, to prevent the oxidation of the cuprous salt by air which is very much faster than the oxidation of ferrous salts.

Very good results have been obtained by following the procedure described below.

To 10 ml of a solution containing between 0.0100 to 0.1000 g. of copper taken in 250 ml conical flask add 10 ml concentrated hydrochloric acid, mix well and then add stannous chloride solution (15 gram  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  dissolved in 100 ml 6 N HCl) drop by drop till the solution becomes colourless and 2 or 3 drops in excess. Add 1 to 1.5 g sodium bicarbonate followed immediately by 5 ml of a saturated solution of mercuric chloride. Close the flask with an air-tight stopper as soon as the evolution of carbon dioxide stops.

Shake gently and wait for 5 minutes for the oxidation of the excess  $\text{SnCl}_2$  by  $\text{HgCl}_2$  to go to completion. Remove the stopper and immediately add about 0.5 g sodium bicarbonate followed at once by 20 ml of an approximately N 10 ferric sulphate solution. No air should enter the flask till after the addition of the ferric sulphate solution. Shake and add 3 ml of phosphoric acid and 3 to 4 drops of 0.3% barium diphenylamine sulphonate indicator solution, dilute to 150 ml and titrate against standard potassium dichromate solution (N 20 or N 10). Each ml of N 10 potassium dichromate is equivalent to 0.006354 g of copper.

Some of the typical results obtained are given in Table I.

TABLE I

| Sl. No. | Copper taken (in grams) | Copper found (in grams) | % error |
|---------|-------------------------|-------------------------|---------|
| 1       | 0.0944                  | 0.0937                  | -0.7    |
| 2       | 0.0629                  | 0.0623                  | -1.0    |
| 3       | 0.0472                  | 0.0470                  | -0.2    |
| 4       | 0.0315                  | 0.0318                  | +1.0    |
| 5       | 0.0252                  | 0.0246                  | -2.5    |
| 6       | 0.0189                  | 0.0183                  | -3.0    |
| 7       | 0.0157                  | 0.0152                  | -3.0    |

The accuracy of the method depends on the efficiency with which air is excluded from the flask during the crucial stages as indicated above.

Only the substances which interfere in the determination of iron by the stannous chloride reduction method (Au, Pt, V, MO, W, Sb, As) interfere in this method of copper determination, besides iron. If interfering substances are present a preliminary separation of copper from them will have to be made in accordance with the classical procedures.

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#### EFFECT OF PROTONATION ON THE RESTRICTED ROTATION ABOUT THE EXOCYCLIC C-N BOND OF NUCLEIC ACID BASES

THE stereochemistry and electronic structure of nucleic acid bases have been subjected to intense investigations in the literature<sup>1,2</sup>. The exocyclic C-N bond in these nucleic acid bases possess considerable double bond character<sup>3</sup>. Molecular orbital calculations have been successfully employed to estimate

the barrier heights to rotation about the C-N bond in these derivatives<sup>2</sup>. Although there are studies in the literature on the experimental determination of the structures of the protonated nucleic acid bases, molecular orbital calculations have not been carried out to understand the observed properties. Thus, it is known that adenine is protonated at the N<sub>1</sub> position in the monoprotonated adenine derivatives such as adenine hydrochloride<sup>3</sup>, 5'-adenosine mono-

calculated as a function of the angle of rotation ( $\omega$ ) around the exocyclic C-N bond, the barrier height being the difference in energies between planar conformation ( $\omega = 0^\circ$  or  $180^\circ$ ) and the transition state ( $\omega = 90^\circ$ ). All calculations were carried out with an IBM 7044/1401 computer.

Our results of the CNDO/2 calculations on the protonation of N<sub>1</sub>-methylcytosine, N<sub>9</sub>-methyladenine and guanine are given in Table I. It is known from

TABLE I  
CNDO/2 Calculations on neutral and protonated bases<sup>a</sup>

|  | E <sub>T</sub> , a.u. | E <sub>a</sub> , kcal mol <sup>-1</sup> | CNDO charges   |                |                |                |                |
|--|-----------------------|---|----------------|----------------|----------------|----------------|----------------|
|  |                       |   | C <sub>2</sub> | C <sub>4</sub> | C <sub>6</sub> | N <sub>1</sub> | N <sub>3</sub> |
| N <sub>1</sub> -Methylcytosine ..              | -93.6225              | 19                                      | -0.403         | -0.322         | -0.172         | -0.140         | -0.342         |
| N <sub>1</sub> -Methylcytosine ..              | -94.1692              | 26                                      | 0.432          | -0.394         | -0.233         | -0.109         | -0.198         |
| N <sub>3</sub> protonated                      |                       |   |                |                |                |                |                |
| N <sub>9</sub> -Methyladenine ..               | -106.0331             | 16                                      | -0.207         | -0.204         | -0.276         | -0.289         | -0.245         |
| N <sub>9</sub> -Methyladenine ..               | -105.4881             | 18                                      | -0.302         | -0.106         | -0.365         | -0.076         | -0.108         |
| N <sub>1</sub> and N <sub>7</sub> diprotonated |                       |   |                |                |                |                |                |
| Guanine ..                                     | -115.9589             | 16                                      | -0.387         | -0.211         | -0.355         | -0.222         | -0.332         |
| Guanine, N <sub>7</sub> protonated             | -116.1708             | 19                                      | -0.426         | -0.373         | -0.365         | -0.222         | -0.295         |

TABLE I—(Contd.)

|  | E <sub>T</sub> , a.u. | E <sub>a</sub> , kcal mol <sup>-1</sup> | CNDO charges   |                |                 |                |                |
|--|-----------------------|---|----------------|----------------|-----------------|----------------|----------------|
|  |                       |   | N <sub>4</sub> | N <sub>7</sub> | N <sub>10</sub> | O <sub>2</sub> | O <sub>6</sub> |
| N <sub>1</sub> -Methylcytosine ..              | -93.6295              | 19                                      | -0.249         | ..             | ..              | -0.420         | ..             |
| N <sub>1</sub> -Methylcytosine ..              | -94.1692              | 26                                      | -0.201         | ..             | ..              | -0.305         | ..             |
| N <sub>3</sub> protonated                      |                       |   |                |                |                 |                |                |
| N <sub>9</sub> -Methyladenine ..               | -106.0331             | 16                                      | ..             | -0.222         | -0.238          | ..             | ..             |
| N <sub>9</sub> -Methyladenine ..               | -106.4881             | 18                                      | ..             | -0.059         | -0.184          | ..             | ..             |
| N <sub>1</sub> and N <sub>7</sub> diprotonated |                       |   |                |                |                 |                |                |
| Guanine ..                                     | -115.9589             | 16                                      | ..             | -0.148         | -0.250          | ..             | -0.382         |
| Guanine, N <sub>7</sub> protonated             | -116.1708             | 19                                      | ..             | -0.003         | -0.244          | ..             | -0.314         |

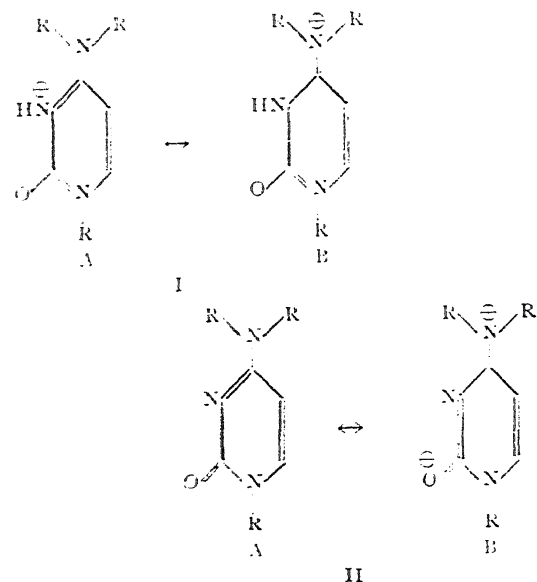
<sup>a</sup> E<sub>T</sub> stands for total energy and E<sub>a</sub>, for barrier height. Only those atoms showing maximum gain or loss of charge have been tabulated.

phosphate<sup>5</sup>, 3'-adenosine monophosphate<sup>6</sup>, adenosine-2'-uridine-5'-phosphate<sup>7</sup>, etc. The only doubly protonated adenine derivative studied has been adenine dihydrobromide which is protonated at N<sub>1</sub> and N<sub>7</sub> positions<sup>8</sup>. Guanine has been reported to be protonated at N<sub>7</sub> position<sup>9</sup>, whereas the site of protonation is N<sub>3</sub> in the case of the cytosine derivative, 3'-deoxycytidinemonophosphate<sup>10</sup>. It was, therefore, considered interesting to carry out a molecular orbital study employing the CNDO/2 method<sup>11</sup> on some of the protonated nucleic acid bases with the primary purpose of evaluating the barrier heights to rotation about the exocyclic C-N bond in these derivatives and to compare them with those reported in the literature for the neutral bases.

The details of the CNDO/2 method employed in our calculations have been described elsewhere<sup>12</sup>. The structural parameters were taken from the literature<sup>8-10</sup>. The energy of the molecule concerned was

the experimentally observed bond distances<sup>11</sup> that the contribution of structure IB to the overall resonance of the cytosine cation is much greater than the contribution of structure IIB to the overall resonance of the neutral cytosine. Accordingly, the barrier heights, E<sub>a</sub>, about the exocyclic C-N bond in all these derivatives increase after protonation, an observation similar to that in secondary amides<sup>12</sup>. Also, the increase in barrier height is greater in N<sub>3</sub>-protonated, N<sub>1</sub>-methylcytosine than in other derivatives. The atoms showing large difference in charge densities on protonation are C<sub>6</sub>, N<sub>1</sub>, N<sub>7</sub> and N<sub>10</sub> in N<sub>9</sub>-methyladenine, C<sub>4</sub>, N<sub>7</sub>, N<sub>10</sub> and O<sub>6</sub> in guanine and C<sub>2</sub>, C<sub>4</sub>, N<sub>3</sub>, N<sub>4</sub> and O<sub>2</sub> in cytosine. These changes in charge densities in the protonated derivatives reflect the differences observed in the structural parameters in these compounds. The barrier heights and charge densities reported here are of significance

as experimental investigations on these systems are not available.



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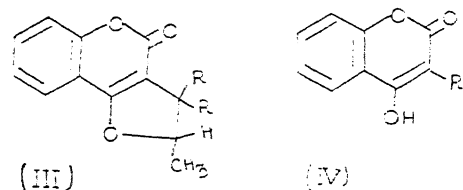
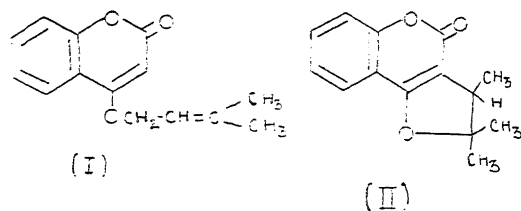
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## ABNORMAL CLAISEN REARRANGEMENT OF 4-PRENYLOXYCOUMARIN

4-PRENYLOXYCOUMARIN (I), was obtained in 7% yield when 4-hydroxycoumarin was heated with 1-chloro-3-methyl-2-butene and potassium carbonate in acetone. The reaction was catalysed by a small amount of potassium iodide. (I), on heating in dimethylaniline, gave a 2,3-dihydro-4-oxo-4 H-2,2,3-trimethyl furo (3,2-c) benzopyran (II) as the only isolatable product. The structure of (II) is confirmed by its NMR spectrum ( $\text{CDCl}_3$ );  $\delta$  1.35, doublet,  $J = 7$  Hz, 3 H at position 3; 1.50, 1.55, two singlets, 6 H of two  $-\text{CH}_3$  at position 2; 3.25, quartet,  $J = 7$  Hz, 1 H at position 3; 7.5, multiplet, 4 H aromatic. If (II) were a normal Claisen migration product, it would have structure (III a), in which case the characteristic quartet of 1 H at position 2 would have appeared in the downfield in the NMR spectrum. Similar type of abnormal Claisen migration has been observed by Jain and Jain<sup>1</sup> during their studies on the Claisen migration of prenyloxy derivatives of phenylbenzylketones.

In view of the above abnormal Claisen migration of 4-prenyloxycoumarin, the Claisen migration of 4-allyloxycoumarin was reinvestigated. 4-Allyloxycoumarin on heating in dimethylaniline gave the normal 2,3-dihydro-4-oxo-4 H-2-methyl furo (3,2-c) benzopyran (III b)<sup>2</sup>. The structure of (III b) is



- (a)  $R = \text{CH}_3$   
(b)  $R = \text{H}$

- (a)  $R = \text{Prenyl}$   
(b)  $R = \text{Allyl}$

confirmed by its NMR spectrum ( $\text{CCl}_4$ );  $\delta$  1.55, doublet,  $J = 7$  Hz, 3 H at position 2; 2.95, multiplet, 2 H at position 3; 5.2, multiplet, 1 H at position 2; 7.25, multiplet, 4 H aromatic. In this

case, the characteristic signal of 1H at position 2 appears downfield. In both the cases, the intermediate migration products, 4-hydroxy-3-prenylcoumarin (IV a) and 4-hydroxy-3-allylcoumarin (IV b) formed are simultaneously cyclized to form the furan ring structure: but however, in the former case the migration is abnormal while in the latter it is normal.

#### EXPERIMENTAL

**4-Prenyloxy coumarin (I).**—A solution of 4-hydroxy-coumarin (1.5 g), potassium iodide (1 g), anhydrous potassium carbonate (3 g) and 1-chloro-3-methyl-2-butene (1 g) in acetone (50 ml) was refluxed for 6 hr. Acetone was distilled off and water was added to the residue, and the whole solution was extracted with ether. Removal of ether gave a solid, which crystallised from petroleum ether, m.p. 98°. Yield 0.2 g (Found: C, 73.26; H, 5.95;  $C_{14}H_{14}O_3$  requires C, 73.04; H, 6.08%).

**2,3-Dihydro-4-oxo-4H-2,3-trimethyl furo (3,2-c) benzopyran (II).**—A solution of 4-prenyloxy coumarin (I) (0.5 g) was refluxed with dimethylaniline (3 ml) for 6 hr the mixture was cooled and added to ice-cold hydrochloric acid. It was extracted with ether and the ether layer was washed with 1% sodium hydroxide solution ( $3 \times 20$  ml). The solid obtained after the evaporation of the ether crystallised from petroleum ether, m.p. 85–86°. Yield 0.4 g. (Found: C, 73.02; H, 6.16;  $C_{14}H_{14}O_3$  requires C, 73.04; H, 6.08%).

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#### SOME OBSERVATIONS ON THE NENCKI REACTION WITH RESORCINOL DERIVATIVES

In the course of our work on the synthesis of aromatic ketones, we happened to study the Nencki reaction on some resorcinol derivatives, when some interesting observations were made which are reported here.

Both *p*- and *m*-resorcylic acids on heating with glacial acetic acid in the presence of anhydrous zinc chloride (2 mol.) for 5–7 minutes underwent decarboxylation and gave resacetophenone in 55 and 50% yields respectively. On the other hand, *o*-resorcylic acid failed to react with acetic acid even in the presence of a large excess of zinc chloride (10 moles), anhydrous aluminium chloride or  $BF_3$  and was recovered unchanged in almost quantitative yields. *p*-Resorcylaldehyde under the conditions of the Nencki reaction afforded a brownish dark solid from which no definite substance could be isolated except the original aldehyde in traces (identified as 2, 4-DNP). Resacetophenone was recovered unchanged in the Nencki reaction with acetic acid under different conditions.

However, both 2-methyl- and 4-ethyl-resorcinols reacted with acetic acid and  $ZnCl_2$  to yield the corresponding ketones, namely, 2, 4-dihydroxy-3-methylacetophenone, m.p. 156° (prepared previously by Hoesch reaction<sup>1</sup>) (2, 4-DNP m.p. above 300°) and (2, 4-dihydroxy-5-ethylacetophenone<sup>2</sup>, m.p. 116°) (2, 4-DNP m.p. 236°) in about 70% yields.

It therefore appears that electron withdrawing groups hinder the reaction whereas electron repelling groups favour it. An exception is orcinol which does not undergo the Nencki reaction.

Also, decarboxylation during the Nencki was not observed in the case of the *o*- and *m*-cresotic acids which were recovered unchanged.

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#### CHEMICAL INVESTIGATION OF CRATAEVA NURVALA, A SEARCH FOR THE ANTI-INFLAMMATORY PRINCIPLE

*Crataeva nurvala* Buch.-Ham. and *C. religiosa* (Capparidaceae) form the source of *Varuna* which finds several uses in Ayurvedic medicine<sup>1,2</sup>. The major constituents of both are lupeol and sitosterol<sup>3,4</sup>. The encouraging anti-inflammatory, anti-arthritis and corticosteroid-like activity<sup>5</sup> of the petrol extract of *C. nurvala* prompted us to reinvestigate the chemical constituents of this extract.

Dried and pulverised root bark of *C. nurvala* (2.5 kg) extracted with petrol (60–80°) furnished a light yellow residue which, on repeated crystallisations from benzene, furnished colourless needles (yield, 0.06%) of  $C_{30}H_{50}O$  ( $M^+$ , 426), m.p.

209–211°, which were found to be identical with lupeol by direct comparison (mixed m.p., superimposable I.R. and CO-TLC). The mother liquor of lupeol on chromatography over Brockmann alumina and elution with pet. ether furnished a solid which crystallised from acetone to give needle-shaped crystals, m.p. 168–169° (yield 0.34%). The compound gave positive test for triterpene with Liebermann-Burchard reagent. It analysed for  $C_{30}H_{48}O$  (M<sub>r</sub>: 424) and showed a carbonyl absorption at 1698  $cm^{-1}$ . The mass spectrum of this compound, besides the molecular ion peak at m/e 424, showed important ion peaks at m/e 409 (M-Me), 381, 218, 205, 189, which indicated its identity with lupen-3-one<sup>6</sup>. This was finally confirmed by its reduction with sodium borohydride to lupeol and by its direct comparison with lupen-3-one obtained by Jones oxidation of lupeol.

Benzene-chloroform and ethyl acetate eluates furnished sitosterol, m.p. 135–136°, and a solid, m.p. 71–72°, which on the basis of IR, NMR and Mass spectral studies and reaction with diazomethane was identified as stearic acid,  $C_{18}H_{36}O_2$ , admixed with  $C_{20}$ ,  $C_{22}$ ,  $C_{24}$  and  $C_{26}$  fatty acids.

Pharmacological investigation<sup>7</sup> with different fractions of *C. nurvala* revealed that the anti-inflammatory property of the total petrol extract is neither due to lupeol nor lupen-3-one, but due to an oily liquid, the least polar fraction of the petrol extract. This oil on careful chromatography over air-dried silica gel G plate showed four overlapping spots (solvent system, P.E. :  $C_6H_6$ , 4 : 1) indicating the complexity of the mixture. Attempts for resolution of this mixture by column or preparative TLC were unsuccessful. The mixture showed positive L-B test for sterol and its I.R. spectrum showed an ester carbonyl band at 1742  $cm^{-1}$ . In accordance with this observation, the oil on alkali hydrolysis furnished an acid ( $\nu_{max}$  1715  $cm^{-1}$ ) and an alcohol ( $\nu_{max}$  3400  $cm^{-1}$ ) both of which were found to be mixtures by TLC studies. The low yield and the complexity of this mixture necessitate combined GLC-Mass spectral studies. Work in this direction is in progress.

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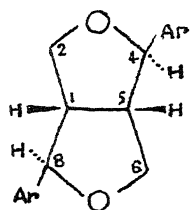
#### LIGNANS FROM THE STEM BARK OF *ZANTHOXYLUM ACANTHAPODIUM* DC.

*Zanthoxylum acanthopodium* DC (family Rutaceae) is valued as a medicinal plant<sup>1</sup>. Two flavonoids, tambulin<sup>2</sup> and tambuletin<sup>2,3</sup> were isolated from the fruits. Kumar *et al.*<sup>4</sup>, reported the presence of linalool, dipentene, phellandrene and citral in the oil of crushed seeds. This report deals with the isolation and identification of the lignans of the stem bark.

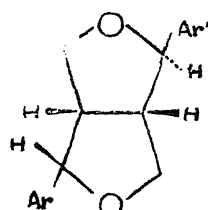
The powdered stem bark was extracted with petroleum ether and the extract on evaporation left a yellowish brown residue. The residue turned partly crystalline after a week. The crystalline solid was separated from the residue by dissolving it with benzene-petroleum ether and filtering. The filtrate was concentrated *in vacuo*, chromatographed over silica gel and eluted with solvents of increasing polarity. The fractions were purified by repeated crystallisations until the compounds were homogeneous in TLC. Six pure neutral lignans were obtained of which three, m.p. 174–77°, 193–95° and 119–20°, were obtained in very small yields. The other three were marked A, B and C.

Compound A (I) obtained from benzene-chloroform (9 : 1) eluate, crystallized as white needles from petroleum etherbenzene, m.p. 123–24°,  $[\alpha]_D^{25} + 64.8^\circ$  (CHCl<sub>3</sub>),  $\lambda_{max}^{E:OH}$  237 and 287 nm,  $\nu_{max}^{CHCl_3}$  2870, 1615, 1490, 1345, 1100, 1060, 1025, 940 and 860  $cm^{-1}$ . It gave positive Labat test (green colour) for methylenedioxy group and analysed for  $C_{20}H_{18}O_4$ . From these properties it was identified as (–)-sesamin and the identity was confirmed by comparison with an authentic sample (mmp and Co-TLC).

Compound B (II) was obtained from benzene-chloroform (3:1) and (1:1) eluates. It crystallised as white plates from petroleum ether-benzene, m.p. 159–41°.  $\lambda_{\text{max}}^{\text{EtOH}}$  236 and 285 nm.  $\nu_{\text{max}}^{\text{KBr}}$  1610, 1590, 1230, 1025, 930, 810 and 720  $\text{cm}^{-1}$ . It analysed for  $\text{C}_{21}\text{H}_{22}\text{O}_5$  ( $M^+$  370) with two methoxys.



I, Ia



II, IIa

- I Ar = 3,4-methylenedioxy phenyl .. sesamin  
 Ia Ar = 3,4-dimethoxy phenyl .. eudesmin  
 II Ar = 3,4-dimethoxyphenyl  
 Ar' = 3,4-methylenedioxyphenyl } fargesin  
 IIa Ar = 3-methoxy-4-hydroxy phenyl  
 Ar' = 3,4-methylenedioxy phenyl } pluviatilol

On permanganate oxidation, it afforded a mixture of veratric acid and piperonylic acid. The presence of dimethoxyphenyl and methylenedioxy groups was also indicated in the n.m.r. spectrum (see Table I). The n.m.r. ( $\text{CDCl}_3$ ) and mass spectra showed that it is a lignan containing a 3,7-dioxabicyclo[3.3.0]octane skeleton. Two doublet signals at  $\delta$  4.8 ( $J = 5$  Hz) and  $\delta$  4.4 ( $J = 7$  Hz) suggested the presence of *cis-trans* configuration in the compound<sup>5</sup>. The striking similarity of the upfield portion of the spectrum of the compound to that of epipinoresinol dimethyl ether also suggested the presence of *cis-trans* configuration. Mass spectral pattern of the compound shows in addition to the molecular ion,  $m/e$  340 (2.5); 220 (4); 204 (9); 219 (9); 203 (23); 184 (2); 168 (2.5); 177 (45); 161 (23); 166 (25); 150 (21); 165 (40); 149 (82); 151 (34); 135 (44); 137 (5); 121 (11); 131 (17); 150 (21); 160 (4) and 178 (16). The spectral data closely agree with that of methyl pluviatilol<sup>6</sup>, m.p. 133–34°.  $[\alpha]_D^{25} - 122^\circ$  which was obtained by methylation of pluviatilol (IIa), a phenolic lignan reported to occur in *Zanthoxylum pluviatilifolium*<sup>6</sup>. Fargesin, m.p. 139°, recently reported as a new natural lignan from the flower buds of *Magnolia fargesii*<sup>7</sup> has also been assigned the same structure. The optical rotation of fargesin has not been cited, but presumably it is racemic. Thus fargesin can be considered as racemic methyl pluviatilol. The specific rotation of compound B is  $+1^\circ \pm 0.5^\circ$  ( $\text{CHCl}_3$ ), suggesting that it is chiefly racemic methyl

pluviatilol or fargesin. This is the second report of the occurrence of this lignan in nature.

TABLE I  
NMR Spectral data of compounds B & C

| Assignment of protons   | Compound B (fargesin) | Compound C (eudesmin) |
|-------------------------|-----------------------|-----------------------|
| 1 H                     | 2.85 m                | 3.08 m                |
| 5 H                     | 3.3 m                 |                       |
| 2 H                     | 4.8 d ( $J = 5$ Hz)   | 4.78 d ( $J = 4$ Hz)  |
| 6 H                     | 4.41 d ( $J = 7$ Hz)  |                       |
| 4 H                     | 3.3 m                 | 4.17–4.4 m            |
|                         | 3.6–4.02 m            |                       |
| 8 H                     | 3.06–4.02 m           | 3.8–4.0 m             |
|                         | 4.02–4.2 m            |                       |
| $\text{OCH}_3$          | 3.84 s                | 3.86, 3.88            |
| $\text{O}_2\text{CH}_2$ | 5.95 s                | ..                    |
| Aromatic                | 6.78–6.85 m           | 6.85–6.9 m            |

Compound C (Ia), obtained from chloroform-methanol (9:1) eluate, crystallised as white needles from petroleum ether-benzene, m.p. 91–92°.  $[\alpha]_D^{25} 0^\circ$  ( $\text{CHCl}_3$ ).  $\lambda_{\text{max}}^{\text{EtOH}}$  234 and 284 nm,  $\nu_{\text{max}}^{\text{KBr}}$  1590, 1520, 1260, 1240, 1025 and 835  $\text{cm}^{-1}$ . The molecular formula ( $\text{C}_{22}\text{H}_{24}\text{O}_5$ ) was assigned from elemental analysis and mass spectrum ( $M^+$  386) [ $m/e$  386 (100), 356 (3), 220 (11), 290 (26), 194 (18), 177 (89), 166 (48), 165 (96), 151 (63) and 134 (4.5)]. On  $\text{KMnO}_4$  oxidation it gave only veratric acid. This showed the presence of dimethoxyphenyl group in the compound. It was confirmed from the n.m.r. spectrum (Table I). A doublet integrating for 2 protons at  $\delta$  4.78 ( $J = 4$  Hz) suggested the presence of diequatorial of *trans-trans* configuration in the compound. These results are in accord with the results of Weinges<sup>8</sup> who reported that a doublet at  $\delta$  4.8 ( $J = 4$  Hz) in sesamin was an indication of *trans-trans* configuration. The spectral data of the compound is in agreement with pinoresinol dimethyl ether (eudesmin). The m.p. and optical rotation indicate that compound C is ( $\pm$ )-pinoresinol dimethyl ether.

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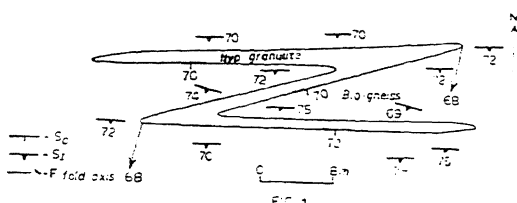
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### SYN-FOLIATION RECLINED FOLDS FROM EASTERN GHAT BELT ROCKS OF PALGHAT DISTRICT, KERALA STATE

Two generations of folds in different scales on foliation are commonly recognised in parts of the Eastern Ghat belt, viz., an earlier generation ( $F_1$ ) running parallel to foliation trend and a later generation ( $F_{11}$ ) of cross folds with their axial traces either perpendicular or at high angle to the former<sup>1-2</sup>.

A third generation of folds ( $F$ ) older than the two mentioned above and with which the formation of foliation is associated in time has been observed recently by the authors during field investigations.

Northwest of Alathur ( $10^\circ 39' : 76^\circ 32' 30''$ ) along the Gayathri river bed two tightly appressed reclined folds with a southerly plunge are formed by a one m thick hypersthene granulite band in biotite gneiss (Fig. 1). The granulite band pinches



off suddenly into the surrounding biotite gneiss east and west of the fold closures. As there is only minor variation in the attitude of the contact ( $S_0$ ) between the granulite band and the gneiss in fold limbs, folding is isoclinal in effect. Parallel to the E-W trending axial surfaces of these folds megascopic foliation (axial surface foliation) is observed in biotite gneiss due to planar preferential arrangement of biotite flakes. While  $S_0$  surfaces curve round the fold closures, foliation surfaces ( $S_1$ ) cut

across  $S_0$  in fold closures. This relationship clearly shows that  $S_0$  is a pre-foliation planar surface that was present in rocks at the time of  $F$  folding and  $S_1$  is a later planar surface formed by secondary metamorphic crystallisation. Further, since  $S_1$  is axial surface foliation with respect to  $F$  folds and has not been affected by  $F$  folding, both  $F$  folding and generation of  $S_1$  are broadly synchronous.  $F$  folds are therefore syn-foliation folds and the metamorphic peak as indicated by the formation of foliation ( $S_1$ ) coincides in time with  $F$  folding.

The syn- and pre-foliation deformational history of the Eastern Ghat belt metamorphic rocks of Kerala and neighbouring areas is little known. Recognition of syn-foliation folds establishes that the major metamorphic event which is synchronous with  $F$  folding must have evidently pre-dated the commonly recognised  $F_1$  and  $F_{11}$  folding episodes in these rocks.

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### BREEDING OF SILVER CARP HYPOPHthalmichthys MOLITRIX (C. AND V.) AND GRASS CARP CTENOPHARYNGODON IDELLA (VAL.) IN A BUNDH TYPE TANK IN WEST BENGAL

IN 1959 Chinese carps, i.e., silver carp, *Hypophthalmichthys molitrix* (C. & V.) and grass carp, *Ctenopharyngodon idella* (Val.) were introduced into India by the Central Inland Fisheries Research Institute, Barrackpore<sup>1</sup>. They are compatible with Indian major carps and thus their culture along with them has been tried by various workers as composite fish culture to maximise fish production in pond.<sup>2-4</sup> Because of their phenomenal growth they are in great demand for composite fish culture. But great difficulty is experienced in obtaining the desired quantity of seed of these carps for stocking as the fishes do not naturally breed in ponds. Even after getting the injections of pituitary hormone unlike Indian major carps they require stripping.

For massive fish seed production, the Indian major carps are bred in perennial as well as seasonal impoundments locally known respectively as wet and dry bundhs in certain districts of West Bengal and Madhya Pradesh<sup>2</sup>. The usual procedure prevalent now in West Bengal is to release the fish in quantity in bundhs after injecting some of them with pituitary extract. The breeding takes place within six hours of injection. An attempt was therefore made to breed silver carp and grass carp in dry bundhs at Simlapal, Bunkura District, West Bengal, during the month of July, 1974. On 7th July, 1974, one female and two males of silver carp were injected @ 9 mg/kg and 4 mg/kg body weight of the fishes respectively and put in a *hapa* fixed in a bundh so as to see the effect of the pituitary injection on the gonad of the female fish while it is in the bundh. Water was allowed to flow in and out of the bundh through the inlet and outlet. A considerable number of eggs has been found already regressed when the female was stripped the following morning. Even so, about 50,000 eggs could be fertilised and their development proceeded upto the formation of embryo after which all of them died. On 30th July 1974, four sets, two of grass carp (consisting of 2 females and 3 males) and two of silver carp (consisting of 2 females and 5 males) were used in the experiment. The female fishes were released in a ditch (10' x 12' x 1') by the side of the bundh after giving a first dose of pituitary extract injection @ 3 mg/kg body weight at 17 hours. Males did not receive any injection at that time. Second injection @ 10 mg/kg, to the females was given at 20-30 hr when males were also injected @ 7 mg/kg and then both the females and males were released in a small dry bundh measuring 50' x 20' with a sloping bottom having a water depth ranging from 6 inches to 1½ feet. One of the females of grass carp died on being released in the bundh, since it slipped from the net and got injured at the time of injection. The inlet and the outlet of the bundh were opened after the release of fish. The water was allowed to flow in from a bigger tank of about 2 hectares containing rain water. This was continued upto 03-00 hr on 31st July, 1974, when both the inlet and the outlet were closed. At about 03-30 hr some fishes were seen coming to the surface and moving around. The activity became intense at 04-00 hr but slowed down at 05-00 hr. At about 05-30 hr, a small piece of mosquito net was dragged and from deeper portion some swollen eggs were recovered. The fishes again came to surface at 06-00 hr and started moving to and fro in the bundh as if searching for food. At 06-30 hr, a pair of silver carp was seen moving together

and sometimes bending very close to each other. At the same time, the grass carp male was seen following the female and nudging the female off and on. At 06-45 hr grass carp courtship could be observed. The male was seen bending over the female and clasping it. A sound of 'chap chap' was produced by the female fish by the brisk movements of its tail. Sex play in grass carp has never been seen or reported, yet the same was clearly observed from 06-30 hr to 09-00 hr in the present experiment in the bundh. Some eggs were collected at about 11-00 hr and kept in enamel trays to watch the development of the zygote. Blastodisc had already been formed in most of the eggs by that time. The percentage of fertilization was about 50%.

The collection of eggs from the bundh was started when the embryo had already been differentiated at 15-00 hr on 31st July with mosquito net *gamcha*. A total of 3.5 lakhs of eggs were collected and kept in hatching *hapas* and earthen pits for hatching. The eggs started hatching from 19-00 hr which continued upto 23-00 hr. After the removal of eggs from the bundh the fishes were examined and it was seen that the female grass carp and one of the two silver carp females fully bred. The experiments clearly indicate that Chinese carps like Indian major carps can breed in bundh type tank and thus the bundh breeding appears to be the solution for mass breeding of these carps so as to meet the ever increasing demand of their seed.

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# EFFECT OF SALINITY ON THE FREE AMINO ACID COMPOSITION OF THE HEMOLYMPH OF A MARINE CRAB—*OCYPODE PLATYTARSIS*

MARINE invertebrates show a decrease in tissue free amino acid levels during periods of osmotic stress in reduced salinities<sup>1,2</sup>. The changes in the tissue free amino acids result in the alteration of free amino acids content in the body fluid<sup>3</sup>. Hitherto little attempt has been made to study the possible changes of free amino acids in the hemolymph of marine crustaceans during the periods of osmotic adjustment. It was therefore considered worthwhile to investigate the changes in the free amino acid pattern of the hemolymph of supra-tidal marine crab, *Ocypode platytarsis*, by acclimating them to different salinities.

*O. platytarsis* is a euryhaline and a good osmo-regulator<sup>4</sup>. To avoid the influence of size, sex and moult stages in the free amino acids of the blood<sup>5</sup>, in the present study, intermoult male crabs of size 85–95 mm were chosen. They were acclimated by maintaining them in 25‰, 50‰, 75‰ and 100‰ sea water for about 12 days at room temperature during which period they were not fed. Crabs collected from the field with the sand were maintained in the laboratory and were used as control under starvation. The experimental animals were prechilled for 4 minutes before drawing blood<sup>6</sup>. The blood was collected in the pre-chilled centrifuge tubes and the free amino acids were separated using ethanol and chloroform employing ascending paper chromatography. The chromatograms were run with *n*-butanol, acetic acid and water (4:1:1 v/v). Besides 0.1% (wt/vol.) ninhydrin solution in acetone, the following spray reagents were employed: Isatin for proline, Pauly's reagent for histidine and tyrosine, Folin's and  $\alpha$ -nitroso  $\beta$ -naphthol for tyrosine<sup>7</sup>.  $R_f$  values were determined and compared with the standards run simultaneously under identical conditions.

The results (Table I) showed that there is a marked difference in the free amino acid pattern of body fluid of the crabs acclimated to various salinities. Normal crabs (controls) showed the presence of histidine, arginine, glycine, alanine, aspartic acid, tyrosine, valine and phenylalanine while in experimental animals, arginine and aspartic acid were absent whereas proline was present.

In addition, crabs acclimated to various salinities showed differences among them. Crabs acclimated in 100‰ sea water showed glycine and histidine to be dominant while tyrosine was in trace amount. Though lysine and aspartic acid were absent in these crabs as in normal animals, these amino acids, however, were present in crabs acclimated to lower salinities (25‰ and 50‰). On the other hand, there was no difference in the amino acid pattern

TABLE I

Free amino acid pattern of the hemolymph of the marine crab—*Ocypode platytarsis* after acclimation to various salinities

| S. No. | Free Amino acids | <i>Ocypode platytarsis</i> |          |          |          |           |
|--------|------------------|----------------------------|----------|----------|----------|-----------|
|        |                  | Normal                     | 25% S.W. | 50% S.W. | 75% S.W. | 100% S.W. |
| 1      | Alanine          | ..                         | ..       | ..       | ..       | ..        |
| 2      | Arginine         | ..                         | ..       | ..       | ..       | ..        |
| 3      | Aspartic acid    | ..                         | ..       | ..       | ..       | ..        |
| 4      | Cystine          | ..                         | ..       | ..       | ..       | ..        |
| 5      | Glutamic acid    | ..                         | ..       | ..       | ..       | ..        |
| 6      | Glycine          | ..                         | ..       | ..       | ..       | ..*       |
| 7      | Histidine        | ..                         | ..       | ..       | ..       | ..*       |
| 8      | Lysine           | ..                         | ..       | ..       | ..       | ..        |
| 9      | Phenylalanine    | ..                         | ..       | ..       | ..       | ..        |
| 10     | Proline          | ..                         | ..       | ..       | ..       | ..        |
| 11     | Tyrosine         | ..                         | ..       | ..       | ..       | (---)     |
| 12     | Valine           | ..                         | ..       | ..       | ..       | ..        |

..: Present; (..): Very feeble; —: Absent;

\*: Increased intensity.

of the hemolymph of crabs acclimated to 25‰ and 50‰ sea water. In 75‰ sea water acclimated crabs, lysine was absent.

These results reveal that the changes in the pattern of free amino acids are in general agreement with the earlier reports on other marine invertebrates<sup>1-3</sup> in which the total quantity of free amino acids of the body fluid increases during acclimation at reduced salinities. This has been suggested as due to fall in the tissue free amino acid level in order to balance the osmotic stress at reduced salinities<sup>3</sup>. The present study supports the concept that there is a correlation between the habitat and the free amino acid composition of the body fluids.

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# A NEW RECORD OF THE CLUPEID FISH, *ILISHA KAMPENI* (WEBER AND de BEAUFORT) FROM THE BAY OF BENGAL

*Ilisha kampeni* (Weber and de Beaufort, 1913)<sup>1</sup> was originally described as *Pellona kampeni* from Java and Borneo. Norman (1923)<sup>2</sup> recorded the species from Madras, based on a single specimen in the British Museum, but this fish has been identified as *I. megaloptera* (Swainson, 1839) by Whitehead (in litt.). Based on this fact Whitehead (1970)<sup>3</sup> equated *I. kampeni* with *I. megaloptera*. *Ilisha kampeni*, which seems to have escaped the attention of ichthyologists all these years, is now recorded for the first time from the Indian waters: Ramaiyan, Natarajan and Whitehead (in preparation) describe further specimens from Porto Novo. The anomalous Porto Novo specimens mentioned by Whitehead (1973)<sup>4</sup> are also *I. kampeni*.

**Description:** based on 95 specimens, 72–148 mm SL. Kakinada, Andhra Pradesh, 11 June 1970.

Branchiostegals 6. D 16–18, P 14–16, V 7. A 42–45, gillrakers 10–11–21–23, scutes 18–20–8–9 (total 26–28); scales in median lateral series 38–40. In percentages of SL: body depth 26.2–29.5, head length 25.8–27.6, snout length 6.9–8.7, eye diameter 6.9–8.7, pre-dorsal distance 45.2–49.2.

Body compressed, its width  $3\frac{1}{2}$  times in its depth, deepest under dorsal origin. Belly keeled, scutes beginning at isthmus, not as troncotent and prominent as in *I. megaloptera* in the post-pelvic region. Maxilla reaching to below middle of eye. No hypomaxilla. Two supramaxillae. Frontals with two prominent ridges of modified *megaloptera* pattern (see Seshagiri Rao, 1972).

Pseudobranch present, basal half covered by a thin membrane (exposed in other species). Dorsal origin a little nearer snout tip than to caudal base. Pectorals almost reaching pelvic base or failing to reach pelvic base by  $\frac{1}{2}$  eye diameter. Anal origin below 8–10 branched dorsal ray.

Swimbladder with paired post-coelomic extensions. **Colour:** Dorsal profile dark gray, upper 1.3rd of flanks light brown with greenish tinge, remainder of flanks silvery white. Posterior half of dorsal fin dark gray at finary tips. Pectorals, pelvics and anal hyaline. Margin of caudal dark.

**Identification and synonymy.**—Considerable confusion exists in the identification of species of *Ilisha*. The form of the swimbladder has recently been found to be an important diagnostic character (Talwar and Whitehead<sup>5</sup>, Seshagiri Rao<sup>6,7</sup>). However, the form of swimbladder in the types of *I. kampeni* had not hitherto been described. Having accepted that Norman's Madras specimen of *I. kampeni* was *I. megaloptera*,

and in the absence of any subsequent record of the former species from the Indian Ocean, I described a new species *I. whiteheadi* from Kakinada which differed from *I. megaloptera* in having a swimbladder with paired post-coelomic extensions (vs. single, present on right side of body only in *I. megaloptera*). Subsequent to the acceptance of the above paper, Whitehead examined Weber and de Beaufort's specimens and informed me that the types of *I. kampeni* have a swimbladder form similar to that in *I. whiteheadi*. It is now presumed that *I. whiteheadi* is a junior synonym of *I. kampeni*; the meristic and morphometric figures given here agree with those in my description of *I. whiteheadi* and with those of the types of *I. kampeni* (Table I).

TABLE I

Comparison of meristic and morphometric characters of syntype of *Pellona* (= *Ilisha*) *kampeni* from ZMA, Amsterdam with specimens from Kakinada and Porto Novo, India

| Character       | 4 syntypes of <i>Pellona kampeni</i> ZMA, 100.0–118.5 mm SL* | 95 specimens from Kakinada 72–148 mm SL | 64 specimens from Porto Novo† 68.0–134.3 mm SL |
|-----------------|--|---|--|
| Dorsal rays     | 16–18  | 16–18                                   | 16–18  |
| Pectoral rays   | 14–15  | 14–16                                   | 15–16  |
| Pelvic rays     | 7  | 7                                       | 7  |
| Anal rays       | 37–40  | 42–45                                   | 37–44  |
| Gillrakers      | 10–11  | 10–11                                   | 6–9  |
|                 | 20–21  | 21–23 (25)                              | 23–25  |
| Scutes          | 19–21  | 17–20                                   | 18–21  |
|                 | 7–9  | 7–9                                     | 7–10   |
|                 | (total, 27–30)   | (total, 24–29)                          |  |
| In % of SL      |  |   |  |
| Depth           | 28.2–29.8  | 26.0–29.5                               | 27.35–30.88                                    |
| Head length     | 28.1–29.2  | 25.8–27.6                               | 27.35–32.35                                    |
| Snout length    | 8.6–8.9  | 6.9–8.7                                 | 10.25–11.76                                    |
| Eye diameter    | 8.6–9.6  | 6.9–8.7                                 | 8.54–10.29                                     |
| Upper jaw       | 13.6–14.7  | 12.5–14.2                               | ..   |
| Lower jaw       | 15.6–16.4  | 12.5–14.9                               | ..   |
| Pectoral length | 15.1–16.0  | 14.8–17.1                               | ..   |
| Pelvic length   | 6.0–6.5  | 5.4–6.5                                 | ..   |
| Anal base       | 33.8–38.3  | 34.0–38.0                               | ..   |
| Pre-dorsal      | 49.3–50.4  | 45.2–49.2                               | 49.5–55.88                                     |
| Pre-pelvic      | 45.7–46.5  | 37.0–41.2                               | 46.15–51.47                                    |
| Pre-anal        | 58.7–63.7  | 48.5–55.4                               | 60.68–66.17                                    |

\* 3 syntypes, 100.0–108.9 mm SL, in Zoologisch Museum, Amsterdam No. 112.594, coll. P.N.V. Kampen, 3–5-1906 and 1 syntype, 118.5 mm SL, in ZMA, No. 112–595, coll. Tissot van Patot, Balikpapan Borneo, were examined for me by P. J. P. Whitehead British Museum (Natural History), London.

† Data from Ramaiyan, Natarajan and Whitehead (in press), which includes 13 specimens in the British Museum (N.H.).

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## EFFECT OF STILBESTROL ON THE GLYCOGEN AND CHOLESTEROL CONTENT OF THE RABBIT UTERUS

NATURAL AND SYNTHETIC oestrogenic substances are one of the potent inhibitors of ovulation<sup>1</sup> as well as of implantation<sup>2</sup>. Little is known about the changes which such substances particularly synthetic non-steroidal oestrogens bring about in the biochemical make-up of the mammalian uterus. Such a study becomes more pertinent since these substances are being increasingly utilized in the clinical interception of pregnancy<sup>3</sup>. This report records the effect of stilbestrol on the glycogen and cholesterol content of the rabbit uterus at progressively increasing intervals of time after the drug administration, and forms a part of a study to evaluate the mechanism of action of postcoital antifertility substances.

Stilbestrol was administered to parous non-gravid rabbit does weighing from 2 to 2.5 kg. A total dose of 1 mg per rabbit was injected in three daily fractions contained in 0.1 ml of olive oil per day. This dose is the minimum 100% effective postcoital antifertility dose for the rabbit. Autopsies were made 24 hours, 72 hours and 144 hours after the last injection. Controls were injected with the vehicle alone. At autopsy, the reproductive tract was quickly exposed, the uterine cornu were excised and then freed from all adhering connective and fatty tissue. Preparation of tissue was done throughout in an ice-bath. Quantitative estimation of the uterine glycogen was done by the method of Montgomery<sup>4</sup> and that of cholesterol by the technique of Zak as described by Hawk<sup>10</sup>. The results were statistically analysed by "Student's" *t*-test<sup>5</sup>.

TABLE I

Effect of 1 mg stilbestrol on the glycogen and cholesterol content of the rabbit uterus; mean value in mg/gm tissue  $\pm$  S.E.M.

|                      | No. of animals | Autopsy time in hours after last injection | Glycogen             | Cholesterol          |
|----------------------|----------------|--|----------------------|----------------------|
| Control              | 5              | ..   | 1.71<br>$\pm 0.09$   | 3.59<br>$\pm 0.18$   |
| Stilbestrol treated: |                |  |                      |                      |
|                      | 3              | 24 hours                                   | 3.37<br>$\pm 0.15^*$ | 2.09<br>$\pm 0.11^*$ |
|                      | 3              | 72 ..                                      | 1.69<br>$\pm 0.06$   | 5.14<br>$\pm 0.05^*$ |
|                      | 3              | 144 ..                                     | 1.17<br>$\pm 0.03^*$ | 5.30<br>$\pm 0.13^*$ |

\* Highly significant ( $p < 0.001$ ).

Table I presents the glycogen and cholesterol values of the uterus of the control and stilbestrol-treated rabbits. It is well known that oestrogen stimulation results in an increase in the uterine glycogen among the rodents<sup>4</sup>. It has also been described that glycogen accumulates mainly in the muscle of the myometrium and endometrium to act as an energy source for uterine contraction<sup>5</sup>. It has been suggested that this increase in the uterine glycogen may result from a stimulation of hexokinase by oestrogenic substances<sup>6</sup>. In the rabbit uterus a similar significant increase ( $p < 0.001$ ) in the uterine glycogen content is noticed after 24 hours of stilbestrol administration; there is then a fall in the glycogen value to the control level by 72 hours of drug administration, probably because the influence of the hormone wears off by this time. However, this drop in the uterine glycogen does not stop but further declines significantly ( $p < 0.001$ ) in rabbits examined after 144 hours of treatment.

In the mouse, oestradiol dipropionate does not seem to influence the uterine cholesterol content<sup>4</sup>, while it has also been reported that oestrogen encourages the binding of labelled acetate to cholesterol in the mouse uterus<sup>1</sup>. In the rabbit, stilbestrol treatment results in a significant decline ( $p < 0.001$ ) of the cholesterol content of the uterus after 24 hours of administration. However, after 72 and 144 hours of treatment the uterine cholesterol levels are significantly elevated ( $p < 0.001$ ). Therefore, it is apparent that the uterine cholesterol content of the rabbit is labile to oestrogen. The exact role played by cholesterol in the economy of the mammalian uterus is not clearly understood, however, it may be added that oestrogen may influence the conversion of uterine cholesterol to other compounds

or may stimulate its synthesis from its acetate precursor, resulting in its decrease or increase in the rabbit uterus.

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## URIC ACID SYNTHESIS DURING AESTIVATION IN *PILA GLOBOSA*

AESTIVATION in the snail, *Pila globosa*, is associated with many changes in nitrogen metabolism. Ammonia and urea are the major nitrogen excretory products in its active life<sup>1</sup> which show a decrease on 3 month aestivation<sup>2,3</sup>. Uric acid on the other hand shows a multifold increase in many tissues of the snail<sup>2,4</sup>. There was no change in the ribonucleic acid content in the hepatopancreas during aestivation<sup>5</sup>. But a significant decrease of the protein

content in the hepatopancreas, mantle and foot was observed<sup>1,6</sup>. Needham<sup>7</sup> pointed out that accumulation within a snail of less than 1 mg uric acid/gm wet weight might be a consequence of nucleic metabolism. But in aestivated snail tissue uric acid values were higher than 4 mg/gm wet weight<sup>3,4</sup> indicating an extensive synthesis of uric acid. Bricteux-Gregoire and Florkin<sup>8</sup> showed that uric acid in gastropods is synthesized from simple precursors, viz., carbon dioxide, acetate, formate, glycine, aspartic acid, glutamic acid. Hence it will be more pertinent if some enzymes specially those concerning with the synthesis of uric acid are studied. In the present report xanthine oxidase which catalyses the conversion of xanthine or hypoxanthine to uric acid was studied since it would give precise information about the increased uric acid metabolism during aestivation in *Pila globosa*.

Xanthine oxidase (EC 1.2.3.2) activity in the tissues was estimated by the dye reduction method of Nachlas *et al.*<sup>9</sup> using 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) as the terminal electron acceptor.

It is found that xanthine oxidase activity is not limited to hepatopancreas alone but is also found in mantle, foot, intestine and heart suggesting that all these tissues potentially oxidise hypoxanthine to uric acid. However, the hepatopancreas had the highest level of activity of the enzyme and next being mantle. Thus, the hepatopancreas is the major centre of uric acid synthesis while mantle is next in the synthetic activity.

On aestivation, the xanthine oxidase activity was found to increase by 85% in hepatopancreas, 60% in mantle, 95% in foot, 52% in intestine and 53% in heart. Though per cent increase of the enzyme activity appears to be high in foot, the total enzyme activity level is high in the hepatopancreas and mantle than the other tissues of aestivated animals suggesting that the major sites of uric acid synthesis remain unaltered. The synthesis of uric

TABLE I

*Xanthine oxidase activity in different tissues of active and aestivated Pila globosa*  
(activity expressed in  $\mu$  moles formazan gm wt/hr.)

|                 | Hepatopancreas |            | Mantle      |            | Foot        |            | Intestine   |            | Heart       |            |
|-----------------|----------------|------------|-------------|------------|-------------|------------|-------------|------------|-------------|------------|
|                 | Active         | Aestivated | Active      | Aestivated | Active      | Aestivated | Active      | Aestivated | Active      | Aestivated |
| Mean            | 16.20          | 30.00      | 13.60       | 21.80      | 6.43        | 12.56      | 8.53        | 12.96      | 3.83        | 5.80       |
| SD              | $\pm 1.26$     | $\pm 0.12$ | $\pm 2.67$  | $\pm 2.43$ | $\pm 1.10$  | $\pm 2.64$ | $\pm 0.94$  | $\pm 1.04$ | $\pm 0.91$  | $\pm 1.06$ |
| <i>p</i> -value | $p > 0.001$    |            | $p > 0.001$ |            | $p > 0.001$ |            | $p > 0.001$ |            | $p > 0.001$ |            |

*p*-value indicates level of significance;  
of six independent observations.

SD=Standard deviation.

Values are mean  $\pm$  SD

acid is elevated in the foot, intestine and heart indicating the tendency towards increased input of nitrogenous wastes. The other tissues, viz., stomach, nephridium, ctenidium, penis, testis had no detectable enzyme activity either in active or in aestivated snail. The reported accumulation of uric acid in those tissues may be due to the transport of uric acid biosynthesised in the hepatopancreas and mantle. Uric acid levels increase in the body fluid of 45 days and 90 days aestivated snails<sup>10</sup> suggesting that transport of uric acid is mediated by this medium. Similar mobilization of uric acid has been reported in the prosobranch, *Thais*<sup>11</sup>. The aestivating *Pila globosa* might have found these organs, viz., stomach, penis, testis, ctenidium as convenient sites for accumulation of uric acid since their natural functions cease to operate.

Though the uric acid synthesis was found in the active snail tissues, uric acid could not be the principal nitrogenous end product since ammonia and urea were found to be the major excretory products in the active snail<sup>12</sup>. Since the presence of an active urea cycle is much debated in gastropods<sup>11,12</sup>, urea production in the active snail and in the snail revived after aestivation was suggested to be through uricolysis<sup>3</sup>. The significance of this circuitous pathway for the production of urea is hard to speculate at present. It may be inferred that on aestivation the uricolysis is blocked and the snail stores all the nitrogenous wastes in the form of innocuous uric acid. The immediate survival demand against ammonia and urea toxicity might have made the animal prefer uric acid production though from the free energy point of view it is a costly waste product.

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## BIOTIN DEFICIENCY AND PERMEABILITY CHANGE IN *ASPERGILLUS NIDULANS*

STUDIES conducted in our laboratory have shown marked reduction in the ability to synthesize fatty acids with concomitant increase in the protein content due to biotin deficiency in *Aspergillus nidulans*<sup>1</sup>. Rao and Modi<sup>2,3</sup> demonstrated the change in the fatty acid content of the cell wall-cell membrane fraction and the increased ammonia uptake due to the deficiency of biotin in *Aspergillus nidulans*. Reduction in the fatty acid content as a result of biotin deficiency has been well documented<sup>4,5</sup>. Comparatively, very little is known regarding the phospholipid content in biotin deficient cells. The present communication deals with the effect of biotin deficiency on phospholipid content and its role in uptake of various solutes.

The strain, the medium composition and the cultural conditions used in the present investigation were same as described earlier<sup>6</sup>. Total lipid was extracted with chloroform : methanol (2 : 1 v/v) and was freed from water soluble impurities by the method of Folch *et al.*<sup>7</sup>. Lipid obtained was chromatographed on thin layer plates (0.5 mm thick) of silica gel, H. (E Merck) as described by Yano *et al.*<sup>8</sup>. Total lipids were visualized by heating the plate at 150°C for 45 mins, after spraying with concentrated H<sub>2</sub>SO<sub>4</sub> : saturated solution of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in water (7 : 3 v/v). For identification of each phospholipid, bands were detected with 2'-7'-dichlorofluorescein, scraped off with razor blade and recovered in chloroform : methanol (1 : 2 v/v). Phosphorus, amino group, reducing sugar and choline were detected by Dittmer's reagent<sup>9</sup>, ninhydrin, anthrone and Dragendorff's reagent<sup>10</sup> respectively. Quantitative assay of each phospholipid was performed by estimating the lipid phosphorus by the method of Bartlett<sup>11</sup>. This was also confirmed by the gravimetric analysis.

Uptake studies were carried out as described earlier<sup>6</sup>. Glucose, ammonia, potassium, phosphorus and nitrate ion were estimated by the methods of Dahlqvist<sup>12</sup>, Fawcett and Scott<sup>13</sup>, Loony and Dyer<sup>14</sup>, Fiske and Subba Row<sup>15</sup> and Goldsmith *et al.*<sup>16</sup>, respectively. From the initial values the rate of uptake was calculated.

Preliminary data indicated about 30% loss in the total phospholipid content due to biotin deficiency. No qualitative difference in the lipid synthesized by biotin deficient and normal cultures was observed. When these phospholipids were analyzed quantitatively, it was observed that biotin deficiency caused a major change in the content of phosphatidylinositol, phosphatidylserine, phosphatidylcholine and phosphatidylethanolamine (Table I).

TABLE I

Phospholipid content of biotin deficient *A. nidulans*

| Phospholipid             | Content (% mg) |           |
|--------------------------|----------------|-----------|
|                          | Normal         | Deficient |
| Lysophosphatidylcholine  | 0.400          | 0.380     |
| Phosphatidylinositol     | 0.136          | 0.095     |
| Phosphatidylserine       | 0.220          | 0.172     |
| Phosphatidylcholine      | 0.622          | 0.386     |
| Phosphatidylethanolamine | 0.439          | 0.162     |
| Cardiolipin              | 0.156          | 0.137     |
| Glycolipids              | 0.086          | 0.082     |
| Phosphatidic acid        | 0.109          | 0.110     |
| Neutral lipids           | 0.152          | 0.119     |

The role of biotin in fatty acid synthesis is well established and fatty acids are known to be one of the constituents of phospholipid. Rao and Modi<sup>1</sup> have shown that the fatty acid content decreases due to the deficiency of biotin in this culture. Thus, the effect of biotin deficiency on phospholipid content may be of secondary one, preceded by the inhibition of fatty acid synthesis.

TABLE II

Uptake of various nutrients by biotin deficient *A. nidulans*

| Nutrient   | Uptake rate ( $\mu$ moles/gr dry cells/10 min) |           |
|------------|--|-----------|
|            | Normal   | Deficient |
| Glucose    | 8.33   | 6.66      |
| Ammonia    | 36.50  | 48.50     |
| Nitrate    | 26.40  | 26.45     |
| Phosphorus | 7.10   | 10.03     |
| Potassium  | 6.90   | 9.18      |

Results listed in Table II suggest that during biotin deficiency there is a change in the permeability of the mold. The results have to be confirmed using labelled substrates in short term experiments. Rao and Modi<sup>2</sup> showed increased ammonium ion uptake by biotin deficient culture, compared to that of normal culture of *A. nidulans*. Earlier, we demonstrated the decreased ability to take up glucose due to biotin deficiency in *A. nidulans*<sup>3</sup>. Further studies showed that glucose binding protein is involved in glucose transport in

this culture<sup>4,5</sup>. The role of phospholipids in membrane transport is well established. Several investigators<sup>6-10</sup> have reported that the properties of membrane transport is affected by the fatty acids incorporated into membrane phospholipid. Thus, the data suggest that the decrease in the phospholipid content due to biotin deficiency may be one of the causes for alteration in the permeability to various nutrients in *A. nidulans*.

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#### A NEW ANTIFUNGAL ANTIBIOTIC PRODUCED BY A SOIL STREPTOMYCETE

As a result of an extensive search for new and useful antimicrobial agents that are produced by soil microorganisms, the authors have been able to isolate a strain of *Streptomyces* sp. designated as Ac<sub>2</sub>, which showed wide antifungal activities. The active material was extracted, purified and characterized as a new polyene antifungal antibiotic, A-2.

The organism was grown in shake flasks at 28° C for 5 days in a medium containing sucrose 20.0, NaNO<sub>3</sub> 3.0, KCl 0.32, NaH<sub>2</sub>PO<sub>4</sub> 0.032, and MgSO<sub>4</sub> · 7 H<sub>2</sub>O 0.016 g/l (pH 6.5). The antibiotic was extracted from the culture filtrate with *n*-butanol, the butanol extract was concentrated and chilled. The active material, which separated as a precipitate from the inactive supernatant, was dried and dissolved in methanol. By repeated addition of cold ether to the methanol solution and chilling a precipitate was obtained which was finally dried *in vacuo*. A-2 was obtained as sulphur yellow powder.

The antifungal spectrum of A-2 as determined by agar-cup method of assay, is presented in Table I.

TABLE I  
Antimicrobial activity of A-2 as determined by the  
agar cup method of assay

| Test organisms                     | Minimum inhibitory concentrations (µg/ml) |
|------------------------------------|---|
| <i>Saccharomyces cerevisiae</i> .. | 1.0                                       |
| <i>Candida albicans</i> ..         | 2.0                                       |
| <i>Aspergillus niger</i> ..        | 50.0                                      |
| <i>Curvularia lunata</i> ..        | 2.0                                       |
| <i>Fusarium udum</i> ..            | 15.0                                      |
| <i>Alternaria solani</i> ..        | 12.0                                      |
| <i>Helminthosporium oryzae</i> ..  | 2.0                                       |
| <i>Blastomyces dermatitidis</i> .. | 0.8                                       |
| <i>Sporotrichum scheinkii</i> ..   | 60.0                                      |
| <i>Microsporum gypseum</i> ..      | 80.0                                      |

The substance is homogeneous as found by paper electrophoresis, paper and thin layer chromatographic studies. A-2 is soluble in water, methanol, ethanol and *n*-butanol, insoluble in chloroform, ether, petroleum ether and acetic acid. It is amphoteric in nature, melts at 142° C (decomposition), optical rotation ( $\alpha_D^{25}$ ) = +50° (C 2% methanol). The ultraviolet absorption spectrum in methanol showed maxima at 360, 380 and 402 m $\mu$  indicating a heptaene compound<sup>1</sup>. Micro-analytical data showed C 41.21%, H 7.14%, N 1.45% and O 50.20% (by difference). The infrared absorption spectrum of A-2 indicated the presence of hydroxyl group at 3350 cm<sup>-1</sup>, either an ester or a lactone at 1710 cm<sup>-1</sup> and a polyene system at 1640 cm<sup>-1</sup>. The band at 920 cm<sup>-1</sup> could be attributed to a disubstituted *trans* double bond system.

The antifungal and physico-chemical properties of A-2 indicate that it is a polyene antibiotic and belongs to the group of heptaenes and to the type of non-aromatic heptaenes<sup>2</sup>. Literature was surveyed extensively to determine the identity of A-2. Comparative studies with known non-aromatic

heptaenes such as amphotericin B<sup>3</sup>, candidin<sup>4</sup>, mycoheptin<sup>5</sup> and blimyacin<sup>6</sup> showed that A-2 is different from these antibiotics in its physico-chemical and antimicrobial properties.

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#### VARIETAL TOLERANCE TO LOW PHOSPHORUS CONDITIONS\*

STUDIES to evaluate the rice varieties suitable for low phosphate conditions were carried out in field and greenhouse at the national headquarters of the All India Coordinated Rice Improvement Project (AICRIP). In one such field experiment, 110 rice varieties including both tall and dwarfs were grown on a black clay soil (pH 7.8, O.M. 2.0%, CEC 42 m.eq./100 g soil, Olsen's P 1.4 ppm, P absorption coefficient 1300 mg/100 g P<sub>2</sub>O<sub>5</sub>) well supplied with N, K and Zn. Almost all the varieties failed to establish under the no-phosphorus treatment, whereas those that survived gave appreciable yields, when 90 kg P<sub>2</sub>O<sub>5</sub>/ha were added. IET 3162, IET 3444 bore a few tillers and gave a low yield (computed to be less than 1.2 t/ha) only in one of the three replicates under no-phosphate. H 4, a tall variety from Sri Lanka, known for its resistance to phosphorus deficiency (Ponnamperuma and Castro, 1972) was among those that failed to survive. It can thus be concluded that pronounced varietal differences do not exist for reactions to low available P below a critical level.

Based upon the results of the preceding study, in a follow-up experiment involving twelve varieties, graded levels of phosphorus were added to a similar soil (Olsen's P 1.0 ppm) in cement pots (40 cm × 40 cm × 52 cm deep). The findings of this experiment are as follows:

### Phosphorus Deficiency

Severe phosphorus deficiency symptoms, such as chlorosis along the margins of the lower leaves followed by scorched appearance and drying, reduced tillering and growth, erect spindly leaves with dirty dark green colour were observed in all the varieties, within 10-12 days of planting, in the no-phosphorus as well as 10 kg  $P_2O_5$  ha treatments. Severity of symptoms decreased with the increased level of P application. The intensity of symptoms on 60th day after planting in different varieties was as follows:

Moderate ... IR 1514A-E666, RPA 5929  
Severe ... RPA 5824, IET 3162, RP 4-17,  
Kumar, Pokkali, Pelita 1/1,  
and IET 3444.  
Very severe ... IET 3166, MI 48, and H4.

### Tiller Number and Grain Yield

Data for tiller number, 60 days after planting (Table I), and grain yield (Table II) reveal the following:

TABLE I

Tiller number of certain rice varieties 60 days after transplanting under different levels of phosphorus

| Variety            | Levels of P ( $P_2O_5$ kg ha) |    |    |    |    |
|--------------------|-------------------------------|----|----|----|----|
|                    | 0                             | 10 | 20 | 30 | 40 |
| RPA 5824           | 0                             | 1  | 6  | 5  | 8  |
| RPA 5929           | 0                             | 2  | 4  | 10 | 13 |
| IET 3162           | 0                             | 2  | 5  | .. | 8  |
| IET 2257 (RP 4-17) | 0                             | 2  | 4  | 10 | 7  |
| Kumar              | 0                             | 0  | 2  | 9  | 9  |
| IET 3166           | 0                             | 2  | 4  | 4  | 6  |
| MI 48              | 0                             | 0  | 2  | 2  | 3  |
| Pokkali            | 0                             | 0  | 2  | 5  | 11 |
| H4                 | 0                             | 0  | 0  | 2  | 2  |
| IR 1514 A-E 666    | 0                             | 4  | 16 | 19 | 24 |
| Pelita 1/1         | 0                             | 2  | 3  | 9  | 12 |
| IET 3444           | 0                             | 2  | 6  | 8  | 8  |

TABLE II

Grain yield of certain rice varieties under different levels of phosphorus

| Variety              | Levels of P ( $P_2O_5$ kg ha) |    |     |      |      |
|----------------------|-------------------------------|----|-----|------|------|
|                      | 0                             | 10 | 20  | 30   | 40   |
| Grain yield (g. pot) |                               |    |     |      |      |
| RPA 5824             | 0                             | 0  | 1.8 | 4.1  | 6.8  |
| RPA 5929             | 0                             | 0  | 1.8 | 11.3 | 19.3 |
| IET 3162             | 0                             | 0  | 2.0 | 7.7  | 10.5 |
| IET 2257 (RP 4-17)   | 0                             | 0  | 0.4 | 2.1  | 2.7  |
| Kumar                | 0                             | 0  | 0   | 1.9  | 2.5  |
| IET 3166             | 0                             | 0  | 0   | 0.7  | 2.7  |
| MI 48                | 0                             | 0  | 0   | 0    | 6.5  |
| Pokkali              | 0                             | 0  | 0   | 15.5 | 29.1 |
| H4                   | 0                             | 0  | 0   | 0    | 0    |
| IR 1514 A-E 666      | 0                             | 0  | 8.7 | 9.2  | 11.7 |
| Pelita 1/1           | 0                             | 0  | 0.4 | 1.3  | 5.6  |
| IET 3444             | 0                             | 0  | 0.2 | 1.6  | 4.0  |

1. None of the varieties produced any grain in no-phosphorus treatment.

2. Except IR 1514 A-E 666, no variety produced tiller when 10 kg  $P_2O_5$  ha was applied. None of the varieties, including IR 1514 A-E 666, could produce any grain yield.

3. With 20 kg  $P_2O_5$  ha treatment Kumar, MI 48, Pokkali and H4 failed to tiller. These varieties including IET 3166 did not give any grain yield. On the other hand IR 1514 A-E 666 which produced the highest number of tillers (16 tillers hill) gave the highest grain yield (8.7 g.). Remaining varieties produced 3-6 tillers and gave grain yield varying between 0.2 to 2.0 g.

4. At 30 kg  $P_2O_5$  ha Pokkali gave the highest grain yield followed by RPA 5929 and IR 1514 A-E 666. Pokkali although had less tiller production at 60 days yet produced more grain. This was because of continuous tiller production even after 60 days (Pokkali is a late variety).

IR 1514 A-E 666 on the other hand although had maximum tiller number yet produced less. This is mainly due to its shattering habit.

Remaining varieties except IET 3162 produced less than 5 g. MI-48 and H4 failed to produce any grain.

5. At 40 kg  $P_2O_5$  the trend in tiller production and grain yield were more or less similar to 30 kg  $P_2O_5$  ha treatment. However, MI-48 gave moderate yield at this level, while H4 failed to produce any grain.

The data on symptoms, tiller number and grain yield clearly demonstrated that IR 1514-E 666 and RPA 5929 are the most efficient in this set of varieties in utilizing the small amounts of added phosphorus. While Pokkali produced the highest yield at higher levels of phosphorus, in actual field conditions under community planting, in view of its poor plant type it is not expected to excel the performance of IR 1514 A-E 666 and RPA 5929. IR 1514 A-E 666 has been reported to be tolerant to low phosphorus conditions at IRRI (Ponnamperuma, 1974). But this variety has other defects, such as shattering and susceptibility to tungro virus. RPA 5929, on the other hand, is reported to possess resistance to bacterial leaf blight, stem borer, gall midge, and green leaf hopper, besides being of mid-duration and having medium slender grains and high yield potential (Seshu *et al.*, 1974, in press). H4, a tall variety from Sri Lanka, reported to be resistant to low P (Ponnamperuma and Castro, 1972) was the most susceptible in our tests. H4 has been bred and grown under low pH conditions in Sri Lanka. Its resistance to low phosphorus has been tested by IRRI soil chemists on an acidic soil (Louisiana clay pH 4.7), unlike



(pH 7-8). Phosphorus in acidic soils is present as iron and aluminium phosphates, whereas under our tests which were performed on an alkaline soil alkaline conditions it is chiefly as calcium phosphate. It is possible that H<sup>+</sup> may be capable of utilizing iron and aluminium phosphates resulting in a fair degree of tolerance to low phosphorus under acidic conditions, whereas it may not have capacity to use calcium phosphates. It is, therefore, essential that when varieties are tested for their tolerance to adverse soil conditions, the relevant soil properties have also to be taken into consideration.

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#### A NONPIGMENTED STRAIN OF *XANTHOMONAS AZADIRACHTII* MONIZ AND RAJ. CAUSING LEAF SPOT OF NEEM (*AZADIRACHTA INDICA* A. JUSS)

Neem (*Azadirachta indica* A. Juss) is an important medicinal evergreen tree on which two bacterial diseases, viz., leaf blight and spot caused respectively by *Pseudomonas azadirachtae* Desai *et al.*<sup>2,10</sup>, and *Xanthomonas azadirachti* Moniz and Raj.,<sup>8</sup> have been reported. During February 1972, severe leaf spot was observed on neem leaves near Jodhpur. A creamy white bacterium was isolated on nutrient agar the pathogenicity of which was proved by inoculating both young and old leaves of neem plant by rubbing the inoculum (ca. 108 cells/ml) mixed with carborundum powder and the same bacterium was reisolated from infected leaves.

The first symptom appears on the leaflets as small, translucent, watersoaked spots which later turn light brown to brown. These spots gradually increase in size coalesce and become necrotic from the centre. The coalesced lesions are often delimited by veins and veinlets giving angular appearance.

The infected leaves eventually become chlorotic, dry and defoliate prematurely.

Albino strains of xanthomonads are reported<sup>1</sup> and validity of some of the species has been doubted by Dye<sup>5</sup>. In India Durgapal<sup>3</sup> reported albino strain of *Xanthomonas uppalii* on *Ipomoea muricata* L. while Jindal and Patel<sup>6</sup> and Patel and Jindal<sup>9</sup> have reported albino strain and species of *Xanthomonas ricinicola* on *Ricinus communis* L. and *Xanthomonas pedatii* on *Pedaliu murex* L. respectively. The bacterium under study is identified as a nonpigmented strain of *Xanthomonas azadirachti* Moniz and Raj., 1967, the bacteriological studies of which are given as follows:

The bacterium is rod shaped, gram negative and weakly motile. On nutrient agar the colonies are round with entire margins, smooth, raised, shining and white in colour. The colony characters on GYCA<sup>4</sup> and PDA<sup>1,4</sup> are the same as observed on nutrient agar except that they are brown on GYCA and creamy white on PDA. Growth on potato wedge was slimy, shining and creamy white in colour. This bacterium did not grow on Kado's<sup>7</sup> medium specific for *Pseudomonas* spp. but grows though slowly on Kado's medium specific for *Xanthomonas* spp.

The bacterium did not utilize asparagine as sole source of carbon and nitrogen; produced H<sub>2</sub>S from cysteine; starch hydrolysed; indole, V.P. and M.R. tests negative; gelatin liquefaction slow; catalase positive; did not reduce nitrate; urease test negative with thymol blue indicator; utilized glucose oxidatively; sodium citrate, succinate, acetate and propionate were utilized but not tartrate and lactate. Growth excellent at 28-30° C, good at 25-34° C, poor at 21° C, and no growth at 5-10° C and 40° C.

The bacterium did not produce symptoms when inoculated by carborundum method on any of the following plants: *Cajanus cajan* (Linn.) Millsp.; *Vigna sinensis* Sari.; *Cicer arietinum* Linn.; *Pisum sativum* Linn.; *Zea mays* Linn.; Wheat, Cotton. *Arachis hypogaea* Linn.; *Brassica oleracea* Linn. var. *capitata* Linn.; *Brassica oleracea* Linn.; var. *botrytis* Linn.; *Raphanus sativus* Linn.; *Solanum tuberosum* Linn.; *Lycopersicon esculentum* Mill.; *Citrus aurantifolia* Swingle; *Vitis vinifera* Linn.; *Amaranthus* sp., *Capsicum annuum* Linn.; *Solanum melongena* Linn.; *Spinacia oleracea* Linn.; *Allium cepa* Linn.; *Ficus religiosa* Linn.; *Phaseolus mungo* Linn.; *Cyamopsis tetragonoloba* (Linn.) Taub.; *Psidium guajava* Linn.; *Mangifera indica* Linn.; *Punica granatum* Linn.; *Rosa* sp., *Tecoma* sp., *Phaseolus aureus* Roxb., *Sesamum indicum* Linn.; *Bougainvillea* sp. and *Duranta* sp.

The culture was sent to Dr. J. F. Bradbury, Bacteriologist, C.M.I., Kew, England, who also

identified it as a nonpigmented strain of *Xanthomonas axadiaractii* (Acc. No. B 5643).

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#### STRAINS OF TOMATO RESISTANT TO ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

*Meloidogyne* spp. have become one of the major limiting factors for the successful production of tomato in most of the vegetable gardens. Improvement of crop plants with genetically built in resistance for nematodes is a well-known approach in plant protection research and a few tomato cultivars resistant to several species of *Meloidogyne* have been developed<sup>1,2</sup>. Varietal resistance, being heritable, is more reliable, inexpensive and eliminates pesticidal hazards. With this point in view and to develop resistant strains for nematodes, five hybrids of tomato (closed pedigree) were received from Dr. T. R. Everett, Ford Foundation Consultant in Entomology and were used as source material to develop pure lines.

In order to develop homozygous lines, the hybrids were allowed to segregate in root-knot nematode sick plots for five generations. Selection pressure was applied for both nematode resistance as well as for plants with high yield potential and optimum fruit size. Pedigree record was maintained throughout. Two lines possessing good agronomic characters were selected and tested for root-knot infection both in field and laboratory conditions. Under laboratory conditions, random sample of twenty plants from each line were grown in pots containing steam sterilized soil to which 1000 second stage larvae, obtained from the pure cultures of *Meloidogyne incognita* and *M. javanica*,

were inoculated. The susceptible variety of tomato 'Pusa Ruby' was used as control. The total number of root-knot galls per plant was recorded six weeks after inoculation.

It is apparent from data (Table I), that the selected lines were completely resistant to *M. incognita* and also showed a high degree of resistance

TABLE I  
Reaction of tomato variety/strain to the attack of *M. incognita* and *M. javanica*

| Variety/strain | Mean number of galls per plant |                    |
|----------------|--------------------------------|--------------------|
|                | <i>M. incognita</i>            | <i>M. javanica</i> |
| Pusa Ruby ..   | 206.8                          | 204.6              |
| Strain 1 ..    | 0.0                            | 4.4                |
| .. 2 ..        | 0.0                            | 7.8                |

to *M. javanica*. The concomitant studies made in the field, heavily infested with root-knot nematodes, also showed a very high degree of resistance. Variety Pusa Ruby was heavily infested and the plants were stunted with poor fruit set. In both the selected strains the plants were normal with a good bearing. The resistant lines appear to be very promising.

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#### CARBOHYDRATE CHANGE IN THE HAEMOLYMPH OF *SPODOPTERA LITURA* (FABRICIUS) DURING THE COURSE OF NUCLEAR POLYHEDROSIS VIRUS DISEASE

TREHALOSE, the major blood sugar of the insect haemolymph varies with nutritional state<sup>1</sup>. Marked fall during several days of starvation has been observed in *Bombyx mori* L.<sup>2</sup>. There is no report on the carbohydrate changes in insects due to nuclear polyhedrosis virus (NPV) disease. However, van der Geest and Craig<sup>3</sup> suggested that the decrease in total solids in the haemolymph of NPV infected *Peridroma saucia* (Hubner) could possibly be due to depletion of food reserves (carbohydrates) in the haemolymph.

Six day old larvae of *S. litura* were fed with heavy suspension of polyhedra for 24 hr and fresh food was supplied thereafter. Haemolymph samples were collected at 24 hr interval for a period of 6

days. The total carbohydrate content of haemolymph after deproteinisation was determined by anthrone reagent<sup>1</sup> using *D*-glucose (AR-BDH) as standard and expressed in g/100 ml of haemolymph.

The carbohydrate contents of the haemolymph of normal and virus fed larvae determined during the course of NPV infection are given in Table I.

TABLE I

Carbohydrate levels (g/100 ml) in the haemolymph of normal and diseased larvae of *S. litura*

| Days after infection | Normal (Mean $\pm$ SD) | Diseased (Mean $\pm$ SD) | t test |
|----------------------|------------------------|--------------------------|--------|
| 1                    | 0.150 $\pm$ 0.010 (4)  | 0.150 $\pm$ 0.010 (3)    | NS     |
| 2                    | 0.230 $\pm$ 0.010 (3)  | 0.210 $\pm$ 0.010 (3)    | NS     |
| 3                    | 0.260 $\pm$ 0.022 (5)  | 0.250 $\pm$ 0.017 (5)    | NS     |
| 4                    | 0.610 $\pm$ 0.007 (5)  | 0.380 $\pm$ 0.006 (3)    | **     |
| 5                    | 1.060 $\pm$ 0.160 (5)  | 0.460 $\pm$ 0.024 (5)    | **     |
| 6                    | 1.110 $\pm$ 0.043 (5)  | 0.620 $\pm$ 0.012 (5)    | **     |

NS, Not significant. \*\* Significant at  $P=0.01$ . Figures in parentheses indicate the number of replicates.

Carbohydrate contents increased with age in both normal and virus fed larvae from an initial level of 0.15 g on the first day to 1.11 g and 0.62 g/100 ml, respectively, for normal and virus fed larvae on 6th day. The carbohydrate level remained almost the same for the first 3 days of observation in both the groups. Though, carbohydrate level increased in the virus fed larvae during 4-6 days of observation, the proportionate increase during this period was much lower compared to the normal larvae and was statistically significant.

Reduced glucose levels in the haemolymph were observed in the mollusc, *Biomphalaria glabrata* infected with larval trematode, *Schistosoma mansoni* after 3 weeks. This was attributed in part, to uptake and utilization by the parasite<sup>2</sup>. A similar reduction in trehalose level was reported in parasitised hornworm larvae, *Manduca sexta* (Johnson)<sup>3</sup>.

There appears to be striking correlation between the appearance of symptoms, food consumption and utilization, and carbohydrate levels in the haemolymph of virus fed larvae of *S. litura*. The carbohydrates got reduced in virus fed larvae during 4-6 days. Similarly, food consumption and utilization were reduced only after 3 days of virus infection (Ramakrishnan and Chaudhari, Unpublished).

It is surmised from these observations that reduced carbohydrate level in the diseased haemolymph of *S. litura* during 4-6 days is due to reduced food consumption which in turn results from virus multiplication.

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## MORPHOLOGY AND GERMINATION OF *PTERIS CRETICA* SPORES

IN the study of *Pteris cretica* L. it was observed that this fern has bilateral as well as tetrahedral spores (Figs. 1 and 3) though normally this fern produces only tetrahedral spores. Panigrahi and Dixit (1969) found trilete and monolete spores in *Ophioglossum reticulatum*. These spores occur in separate sporangia which originate from the same sorus. Pant and Srivastava (1962) found trilete and monolete microspores in the same microsporangium in *Isoetes indica*. Occurrence of two different kinds of spores within two distinct types of sporangia (of the same size) is rare. Sporangia contain 40% bilateral spores while 60% are tetrahedral.

Tetrahedral spores (Figs. 1 A, B) are  $39 \times 42 \mu$ . They appear to be subtriangular in lateral view and are somewhat rounded in proximal view. They have a distinct trilete mark, each arm being  $22 \mu$  long. Exine is  $4 \mu$  thick and dark brown in colour. Sexine and nexine are not distinguishable. Exine is rugulose, rugulae appear as crenate projections.

Bilateral or monolete spores are  $55 \times 32 \times 45 \mu$  (Figs. 3 A, B) they are somewhat longer than the trilete ones. They appear to be planoconvex in lateral view and oval in proximal view. Laesura is  $32 \mu$ , the exine is  $4 \mu$  thick and light brown in colour. Exine ornamentation is identical with that of the tetrahedral spores.

Occurrence of both, tetrahedral and bilateral type of spores in *Pteris cretica* has not been reported earlier. Germination of both kind of spores has been studied and is reported below:

Both types germinate after the 5th or the 6th day after sowing. Tetrahedral spores germinate to produce a rhizoidal and a body cell. The latter has numerous chloroplasts (Fig. 2 A). This cell divides transversely a number of times to produce a filament of 4 to 5 cells in 3 days (Figs. 2 B, C, D, E). This filament is comparatively smaller in size as compared to that produced by bilateral spores. The filament remains in this stage for 10 to 12 days. Now, the terminal cell undergoes vertical division (Fig. 2 F). Subsequent divisions form a flat, fan-shaped structure which ultimately

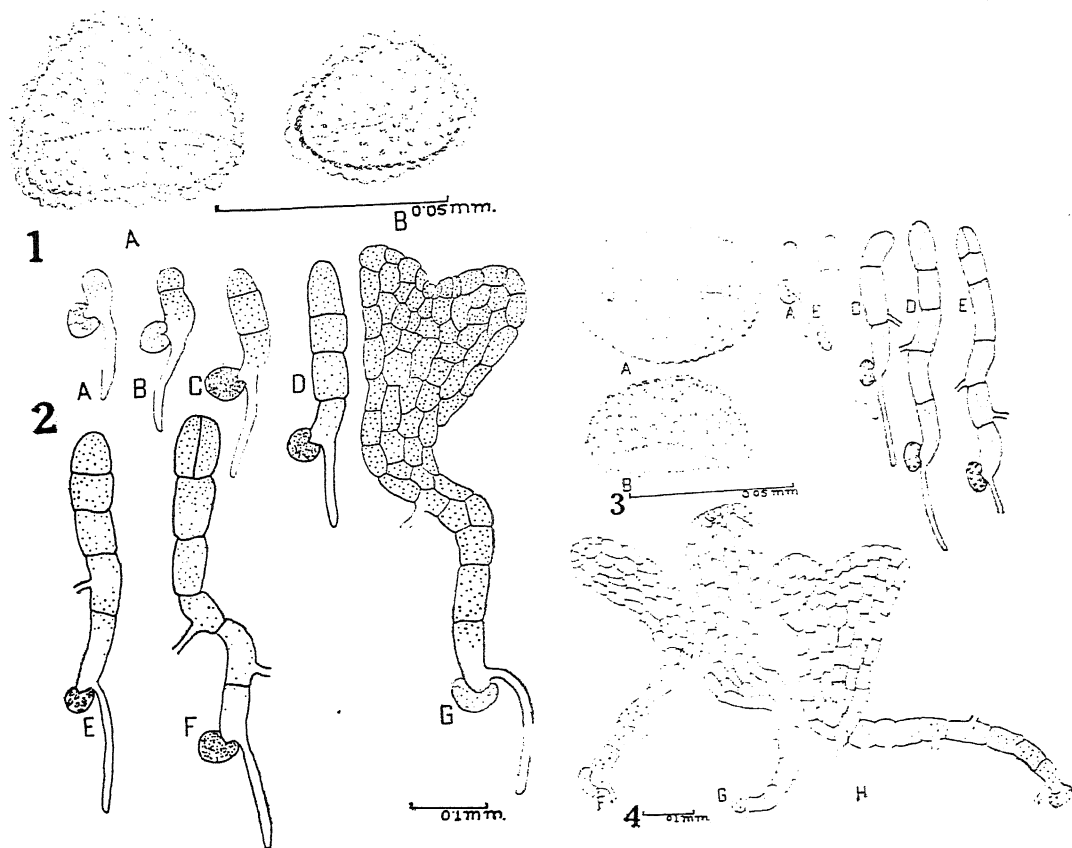
develops into a cordate prothallus (Fig. 2G) with fewer rhizoids as compared with the bilateral spores.

Bilateral spores germinate to produce a rhizoidal and a body cell, the latter is comparatively long (Fig. 4A), 4 to 5 celled stage is reached quickly in 24 hours (Figs. 4B, C, D). The terminal cell divides vertically on the 5th or the 6th day after germination (Fig. 4E). The number of rhizoids produced is more. The filamentous stage gets

changed soon into a cordate prothallus and progresses in vertical divisions (Figs. 4F, G and H).

Monolete spores take 11 days while the trilete ones take 20 days for the formation of a cordate prothallus almost equal size under identical conditions.

It is therefore concluded that the pace of development exhibited by bilateral spores is faster than that shown by the tetrahedral ones.



Figs. 1-4. Fig. 1. A. Trilete spore in lateral view. B. In proximal view. Fig. 2. A-G. Germination of tetrahedral spore of *Pteris cretica* showing stages in the development of prothallus. A-Germination on the 6th day after sowing of the spore showing body cell and a rhizoidal cell. B & C-2nd and 3rd stage of development on the 2nd day after germination. D-4th stage, 4 celled filament after germination on the 6th day. E-5th stage, 5 celled filament after germination on the 6th day. F-6th stage, apical cell of the filament divides vertically on the 10th day. G-7th stage, apical notch is formed and beginning of the usual cordate shaped prothallus on the 20th day after germination. Fig. 3 A-Monolete spore proximal view. B-Lateral view. Fig. 4. A-H. Germination of bilateral spore of *Pteris cretica* showing stages in the development of prothallus. A-1st stage of germination on the 6th day of sowing of spores showing body cell and rhizoid. B & C-2nd and 3rd stage of development on the 2nd day after germination. D-4th stage, filament having many cells and 3 rhizoids on the 3rd day of germination. E-5th stage, terminal wall of the filament showing vertical division on the 6th day after germination. F-6th stage, spatulate stage reached on the 10th day after germination. G-7th stage, spatulate part becomes wider on the 10th day after germination. H-8th stage, apical notch is formed and cordate shape of prothallus becomes discernible on the 11th day after germination.

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### EFFECT OF KINETIN ON THE SEX RATIO IN *DROSOPHILA MELANOGASTER*

CYTOKININS have been shown to induce a shift in sex balance towards femaleness in hermaphrodite flowers<sup>1</sup>, and the male flowers into a hermaphrodite<sup>2</sup>. Other phytohormones like gibberellic acid<sup>3</sup> and ethylene<sup>4</sup> are known to change the balance in sex in plants.

Isopentenyladenosine, a cytokinin, has been shown to promote growth as well as inhibit growth in human cells (lymphocytes) depending upon the concentration<sup>5</sup>. A few other studies also show some physiological effects of cytokinins in animal cells<sup>1</sup>.

The present investigation was carried out to study the formative effects, if any, of kinetin on *Drosophila melanogaster*. Kinetin at three concentrations, viz., 300 ppm, 200 ppm and 100 ppm were incorporated into the corn meal agar media and 20 pairs of male and female of Wild type flies were released into the media. Each treatment was replicated thrice with controls. A concentration of 300 ppm kinetin was toxic and the larvae did not survive. Morphological observations revealed no abnormalities of any kind but for the sex ratio which was 1:1 in all the controls (kinetin-free). But in the kinetin supplemented media the sex ratio of  $F_1$  progeny was 1:1.5 in both treatments with 100 and 200 ppm of kinetin. A similar ratio (1:1.5) in  $F_2$  and  $F_3$  generations was also observed (Table I). To know whether kinetin induced a permanent change in the sex ratio, the progeny from the kinetin supplemented media were transferred to kinetin-free media, and it was observed that the sex ratio reverted back to 1:1. The kinetin induced change in sex ratio was therefore purely temporary and was not heritable.

TABLE I

Population of males and females grown on kinetin supplemented media and controls for generations

|                             | 100 ppm |        | 200 ppm |        | Control |        |
|-----------------------------|---------|--------|---------|--------|---------|--------|
|                             | Male    | Female | Male    | Female | Male    | Female |
| (Average of 3 replications) |         |        |         |        |         |        |
| Generation                  |         |        |         |        |         |        |
| $F_1$                       | 221     | 323    | 257     | 359    | 259     | 279    |
| $F_2$                       | 140     | 266    | 235     | 353    | 251     | 274    |
| $F_3$                       | 190     | 242    | 166     | 238    | 114     | 127    |

$X^2$  values significant for all the three generations at 5% level.

The above results indicate kinetin acts like a phenocopy inducing agent, acting on the sex ratios in *Drosophila melanogaster*.

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### ROOTING RESPONSE OF TEA CUTTINGS TO 'ALAR-85'

It has been reported that N, N'-dimethylamino-succinamic acid ('Alar-85') is promising for promoting rooting of cuttings of several ornamental species. (Read and Haysler, 1970 cited by Wittwer, 1971). In experiments carried out recently (October to December, 1974) we observed that this growth substance is useful in inducing abundant root formation in tea cuttings.

The standard technique of rooting 'leaf and internode' cuttings (Eden and Bond, 1941) was followed. Green wood cuttings of a good rooting tea clone 'B/6/29' were treated with 'Alar-85' at the concentrations of 2,500, 5,000, 7,500 and 10,000 ppm in 50% ethyl alcohol. The quick-dip method was adopted and 4 to 6 mm of the basal ends of the cuttings were immersed in the respective solutions for 5 seconds and were inserted in the rooting medium (sandy loam with a pH of 5.3). In our earlier trials, treating tea cuttings with 50% ethyl alcohol did not show any effect on inducing rooting of cuttings (Venkataramani, K. S., unpublished) and therefore in all our later experi-

TABLE I  
Effect of treatment with 'Alar-85' on rooting of tea cuttings

| Treatment               | % cuttings rooted after |         | Number of roots for 10 cuttings* | Total length of roots of 10 cuttings in mm* | Dry weight of roots of 10 cuttings mg* |
|-------------------------|-------------------------|---------|----------------------------------|---|--|
|                         | 4 weeks                 | 8 weeks |                                  |   |  |
| (1) Control             | .. 10                   | 41.1    | 57                               | 976   | 122                                    |
| (2) 'Alar-85' 2,500 ppm | .. 40                   | 60.0    | 90                               | 2,262                                       | 271                                    |
| (3) 'Alar-85' 5,000 "   | .. 20                   | 51.0    | 52                               | 1,292                                       | 159                                    |
| (4) 'Alar-85' 7,500 "   | .. 10                   | 38.0    | 56                               | 926   | 85                                     |
| (5) 'Alar-85' 10,000 "  | .. 0                    | 39.0    | 44                               | 784   | 90                                     |

\* At the final examination.

ments we have been using only the untreated series of comparison purposes. Each treatment consisted of 100 cuttings. 10 cuttings from each treatment were examined at the end of 4 weeks. When the experiment was terminated at the end of 8 weeks all the remaining cuttings were examined. The results obtained are presented in Table I.

The cuttings treated with 'Alar-85' at the concentration of 2,500 ppm showed a greater percentage of rooting, more number of roots per cutting and a greater number of cuttings rooted earlier than the untreated ones. It was further observed that treatment with 'Alar-85' at this concentration resulted in a better root system (Fig. 1). The chemical inhibited rooting at very high concentrations (7,500 and 10,000 ppm). At 10,000 ppm shedding of the mother leaf occurred in 21% of the cuttings between the 4th and 8th week after treatment.

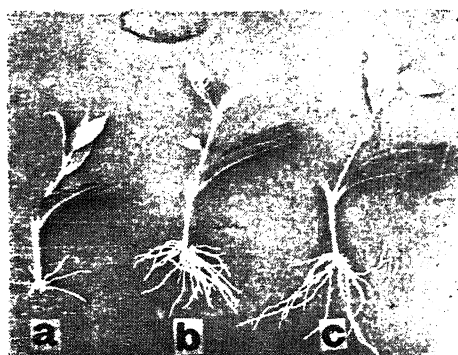


FIG. 1. Effect of 'Alar-85' on rooting of the cuttings of clone 'B/6 29': (a) Untreated. (b) 2,500 ppm. (c) 5,000 ppm.

I am thankful to Dr. K. S. Venkataramani, Director and Dr. V. S. Sharma, Botanist, UPASI Tea Research Station for helpful criticism of the script and for their interest in the investigation.

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### MOSAIC VIRUS OF *ERYSIMUM HIERACHIFOLIUM* LINN.

A SEVERE mosaic disease on the leaves of *Erysimum hieracifolium* Linn. plants was noticed during May 1972 at the Horticultural Research Station, Simla. *E. hieracifolium* is perennial and an important medicinal plant which grows wild at higher altitude ranging from 2,000 metres to 4,000 metres. The characteristic symptom of the disease on the infected leaves showed the presence of green yellow patches which later diffused and entire leaf became discoloured. The leaves exhibited marginal curling and reduction in leaf lamina. The infected plants were stunted as compared to healthy ones. The transmission test by means of seeds procured from diseased plants was unsuccessful. However, this causal agent was successfully transmitted through insect vectors as well as the juice extracted from the diseased leaves establishing the viral nature of disease.

The virus was readily transmitted by the juice extracted from the diseased leaves of *Erysimum* plants which were growing wild in the field. The carborundum 600-mesh was applied on the healthy leaves as an abrasive before the inoculation. A large number of plants comprising of both cruciferous and non-cruciferous were used in the experiments. The plant species which could be infected mechanically were *Brassica chinensis* L., *B. juncea* (L.) Coss., *B. hirta* Moench, *B. integrifolia* O.E. Schultz., *B. nigra* (L.) Koch and *Nicotiana tabacum* L. var. White Burley. However, *Brassica oleracea* L., *Capsicum annum* L., *Chenopodium amaranticolor* Cosse and Reyn., *Cucumis sativus* L., *Datura stramonium* L., *Gomphrena globosa* L., *Phaseolus vulgaris* L., *Raphanus sativus* L., *Spinacea oleracea* L., *Vigna sinensis* Savi and *Zea mays* L., were found to be immune to the virus. The finding

was confirmed by back inoculation from test plants to original host which indicated the absence of the virus infection in the plants inoculated.

The physical properties of the virus indicated that this was inactivated when heated for 10 minutes at 50° C to 55° C. The dilution end point between 1 : 1000 to 1 : 1500 and remained infectious in the sap when stored at 15° to 18° for 10 days.

*Myzus persicae* Sulz., *Brevicoryne brassicae* (L.) and *Aphis gossypii* Glov. were employed for the study of insect transmission of the virus. Both viruliferous nymphs and adults of all three species of aphids were found to be capable of transmitting the disease in non-persistent manner from the infected leaves of *Erysimum* plants to *Brassica chinensis*, *B. nigra* and *B. juncea*. The virus was then recovered from these hosts to *Erysimum* plants by back inoculation.

The present studies of *Erysimum* mosaic virus revealed that the causal agent is transmitted by aphids and also by sap. The host range, physical properties and insect vectors are similar to that of Chinese Sarson mosaic virus as described by Azad and Sehgal<sup>1</sup>. Although slight variations in dilution end point and longevity *in vitro* of the virus were

noticed. It is therefore, concluded that the mosaic virus infecting *Erysimum* plants is related to Chinese Sarson mosaic virus, though it differs slightly in its physical properties. The occurrence of similar virus has also been reported on *Helipterum* (*Acroclinium*) *roseum* by Vashisth<sup>2</sup> causing mosaic symptoms. Since *E. hieracifolium* plant happens to be perennial and natural host it may act as potential source of Chinese Sarson mosaic virus.

Sincere thanks are due to Dr. R. N. Singh, Head, Division of Horticulture and Fruit Technology and to Dr. S. P. Raychaudhuri, Head, Division of Mycology and Plant Pathology, I.A.R.I., New Delhi, for their encouragement and providing facilities. The author is also grateful to Regional Botanist, Botanical Survey of India, Dehra Dun for identification of host plant.

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Current Science Association

## A New Indicator for Direct EDTA Titration of Ferric Ions

Salicylic acid and its related compounds<sup>1</sup>, ortho-hydroxy ketones and their oximes<sup>2</sup>, protocatechuic aldehyde and its related compounds<sup>3</sup>, cinnamohydroxamic acid, *n*-sulfophenyl hydroxamic acid, *n*-benzoyl-*n*-phenyl hydroxylamine and *n*-phenyl hydroxamic acid<sup>4</sup>, 4-OH-1-*p*-sulfonate phenyl triazine<sup>5</sup> and a number of substances have been used as indicators for direct EDTA titration of ferric ions, while aluminon has not been investigated as an indicator. This article reports the use of aluminon as an indicator for the direct titration of ferric salts with EDTA solution.

The stock solutions of ferric nitrate, EDTA and the indicator were 0.1 M, 0.01 M and 1.0% respectively. Solutions of interfering ions were 0.2 M. The Fe<sup>3+</sup> solution was standardised gravimetrically as ferric oxide.

Aluminon gives a violet colour with ferric ions. There is a sharp colour change on titration with EDTA from violet to pale yellow at the end point. When an aliquot of Fe<sup>3+</sup> is titrated with EDTA, iron can be estimated accurately in the PH range 2.0-3.0 with the help of the indicator investigated. Titrations carried out with different concentrations of the indicator, showed that an addition of 2 drops of 1.0% solution of the indicator gave satisfactory results for 5-10 ml of 0.01 M ferric nitrate solution. The titration could be satisfactorily carried out in the temperature range 10-60° C. At 2.8 mg concentration of iron, 50 times excess of Na<sup>+</sup>, K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, 10 times excess of Ni<sup>2+</sup>, Co<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup> and 5 times excess of Ca<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>, Cr<sup>2+</sup> and Cu<sup>2+</sup> could be tolerated. Similar observation with other indicators have been reported by Desai and co-workers<sup>6,7</sup>.

Take 5.0 ml aliquot of 0.01 M ferric nitrate solution, adjust pH 2.0-3.0 using 1-2 ml of acetate-HCl buffer of pH 2.0, add 2 drops of 1.0% solution of the indicator and titrate against 0.01 M solution of EDTA to pale yellow colour.

5.0 ml aliquots of 0.01 M ferric nitrate were analysed according to the suggested procedure. The standard deviation was 1.0%.

Thanks are due to the Principal, Borsad Science and Law College and E.M.H.S. Trust, Borsad, for extending laboratory facilities.

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## Amitrole Toxicity to Crops and its Reversal by Urea

A critical assessment of response of the crops against herbicides is certainly essential. In this experiment pearl millet and cotton seeds were supplied with 2 ml of amitrole 3 amino-*s*-triazole of 10 and 20 ppm respectively. Urea preparations of various concentrations (ppm) were added after two days. Seeds were allowed to grow in petri-dishes and chlorophyll from the first formed leaves was estimated.

It was noted that with 5 ppm of amitrole, millet leaves appeared less green and at 10 ppm became white while in cotton leaves became yellowish at 20 ppm, but no reduction in size of seedlings was noted. At 50 ppm the seedling length was reduced by 13.9% and 7.2% in pearl millet and cotton respectively. The chlorophyll content in millet was reduced to 0.07 mg/g fresh weight in contrast to 4.17 mg/g of the untreated ones. The chlorotic effect could gradually be removed with urea treatment and complete recovery was possible with 500 and 750 ppm in millet and cotton respectively.

Biological properties of amitrole as potent herbicide were described by Behrens (1953). Hall (1954) expressed that lower dosages of amitrole stimulated growth and higher dosages (about 210 to 420 ppm) were chlorotic. The results of the present investigation pointed that even 10-20 ppm of this herbicide can inhibit biosynthesis of chlorophyll in leaves of some crops. Though chlorosis and disorganised chloroplasts are the first symptoms of amitrole injury (Bartels and Weier, 1969) its recovery was not tried. The reappearance of



TABLE I

*Amitroie Toxicity and its Reversal by Urea expressed as Chlorophyll Content*

| Chlorophyll<br>mg/g<br>in crop | Treatment: Conc. (ppm) of amitroils (A) and Urea (U) |           |           |            |            |            | Control |
|--------------------------------|--|-----------|-----------|------------|------------|------------|---------|
|                                | A 10   | A 10—U 10 | A 10—U 50 | A 10—U 250 | A 10—U 500 |            |         |
| Pearl millet                   | 0.07   | 0.14      | 0.93      | 2.67       | 4.12       |            | 4.17    |
|                                | A 20   | A 20—U 10 | A 20—U 50 | A 20—U 250 | A 20—U 500 | A 20—U 750 | Control |
| Cotton                         | 0.62   | 0.97      | 1.9       | 3.1        | 5.2        | 6.39       | 6.33    |

chlorophyll by treatment with urea, thus, appears to be of practical significance. However the fact that seedling length and area of leaves was not at all affected support Putala's (1967) finding that sublethal concentrations may not interfere with differentiation of other cellular organelles.

Authors thankfully acknowledge the suggestions and facilities provided by Prof. L. P. Mall, Head of the institution.

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### Two Leaf Spot Diseases of *Zizyphus* from Haryana

*Zizyphus mauritiana* Lamk. is extensively planted in Haryana for the fruits. During February–March 1974 two leaf spot diseases were collected from Horticulture garden of Haryana Agricultural University, Hissar. The pathogens were fairly widely distributed on the host plant.

The first specimen revealed the formation of small dark-green to brownish spots on the leaves. The fungus was identified as *Cladosporium herbarum* (Persoon) Link. The pathogenicity was proved by inoculating the spore suspension on healthy leaves. It constitutes the first record of its occurrence from Haryana.

Another disease produced spots on the leaves, epiphyllous, yellow at first, then brown with a dark-brown margin, circular to oval, upto 4 mm in diameter, black, conidiophore 15–20 × 4 µ, straight, conidia 40–75 × 4 µ, the smaller one cylindric, the larger one clavate and tapering to 2 µ diameter (in nature) straight, ends obtuse, 3–8 septate, hyaline to olive-green in colour.

The present fungus is identified as *Cercospora zizyphi* Petch. It has also not been reported on *Zizyphus mauritiana* Lamk. from Haryana and constitutes a first record of its occurrence.

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### *Brassica tournefortii* Gouan—A New Host of *Peronospora parasitica* (Pers.) de Bary

*Brassica tournefortii* locally known as *sodia* is grown in some parts of Haryana under rainfed conditions. During February to March 1974 the crop was found to be severely attacked by a downy mildew disease at research farm of Haryana Agricultural University, Hissar. Symptoms were noticed on the leaves, stem and pods in the form of characteristic purplish brown spots. The spots were small in the beginning but covered the whole surface of the leaves towards the end. A snow-white colour growth of the fungus occurs under surface of the leaves.

The fungus is identified as *Peronospora parasitica* (Pers.) de Bary. The present fungus has not been recorded on the above host and hence constitutes a new host record.

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Hissar, December 9, 1974.

### New Host Records of Castor Semi-looper, *Achaea janata* L. (Lepidoptera: Noctuidae)

So far, castor semilooper (*Achaea janata* Linn.) has been reported to attack 26 host plants.

In the months of August and September, 1974, the authors found a large number of castor semilooper larvae feeding on the tender leaves of *Bauhinia purpuria* L., and the flower buds and floral parts of *Phaseolus aurens* L. at the Agricultural College Campus, Dharwar, Karnataka State.

The larvae when reared on plant parts of these new hosts successfully completed their life cycle. The two host plants thus, form new host records of *A. janata*.

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November 26, 1974. T. S. THONIAIDARYA.

***Muraya paniculata* Linn.—A New Host for *Diaphorina citri* Kuwayama**

*Muraya paniculata* Linn., a much valued flowering hedge was found severely attacked at the Landscape Nurseries of the Punjab Agricultural University, Ludhiana, during 1974 by *Diaphorina citri* Kuwayama. The attacked plant had abnormal appearance. The leaflets were yellow, curled and reduced in size. These leaflets dried up later and fell off prematurely. Average population of nymphs was found as high as 93 per leaf and 10–13 per leaflet.

The pest has been reported to attack almost all the species of citrus (Bindra<sup>1</sup>), Murwa (*Muraya koenigii* Speng) (Fletcher<sup>2</sup>) and Wampee [*Clausena lansium* (Lour) Skeels in China (Hoffman<sup>3</sup>)]. *Muraya paniculata* thus, appears to be a new host plant for *Diaphorina citri*.

Department of Horticulture, S. S. CHEEMA.  
Punjab Agricultural University, S. P. KAPUR.  
Ludhiana, December 2, 1974.

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**Association of 'Mudworm' *Polydora ligni* Webster with *Mytilopsis sallei* (Recluz) (Polycypoda)**

Among the several known species of *Polydora*, *P. ciliata*<sup>1-5</sup>, *P. websteri*<sup>6</sup>, *P. ligni*<sup>7</sup> and *P. hoplura*<sup>8</sup> have been frequently encountered as associates in shells of oysters from different countries. Incidence of *Polydora ligni* from Indian waters, however, has not been reported so far.

In the course of studies on the fouling bivalve *Mytilopsis sallei* (Recluz) incident at Visakhapatnam, 'blisters' were encountered in a large number of animals. Out of nearly 200 animals of *M. sallei* examined, about 20 specimens revealed the presence of these blisters which were found to harbour at least one worm each.

Regarding the nature of association of the worms with host some workers attribute oyster mortality to infection by *Polydora* sp. Extensive destruction of oyster beds in Australian waters has been attributed to infection by *P. ciliata* and *P. ligni*<sup>2</sup>. On examination of the nutritive value of infected and healthy oysters, Loosanoff and Engle<sup>2</sup> recorded that *P. websteri* did not cause any serious damage to the oysters in American waters but the same was stated to be responsible for the destruction of oysters in Dutch waters<sup>9</sup>. Presently no harmful effects could be noted in the host animals. It is, therefore, suggested that the association between *P. ligni* and *M. sallei* may be commensalic.

The authors are grateful to Dr. R. Philip Dales, Professor of Zoology, Bedford College, London, for valuable suggestions and critically reading the manuscript. Thanks are also due to Captain P. R. Sen, IN., Director and Sri. S. V. S. Rao, Deputy Director of this Laboratory, for their encouragement.

Naval Science and S. S. GANTI.\*  
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## INFORMATION TO CONTRIBUTORS

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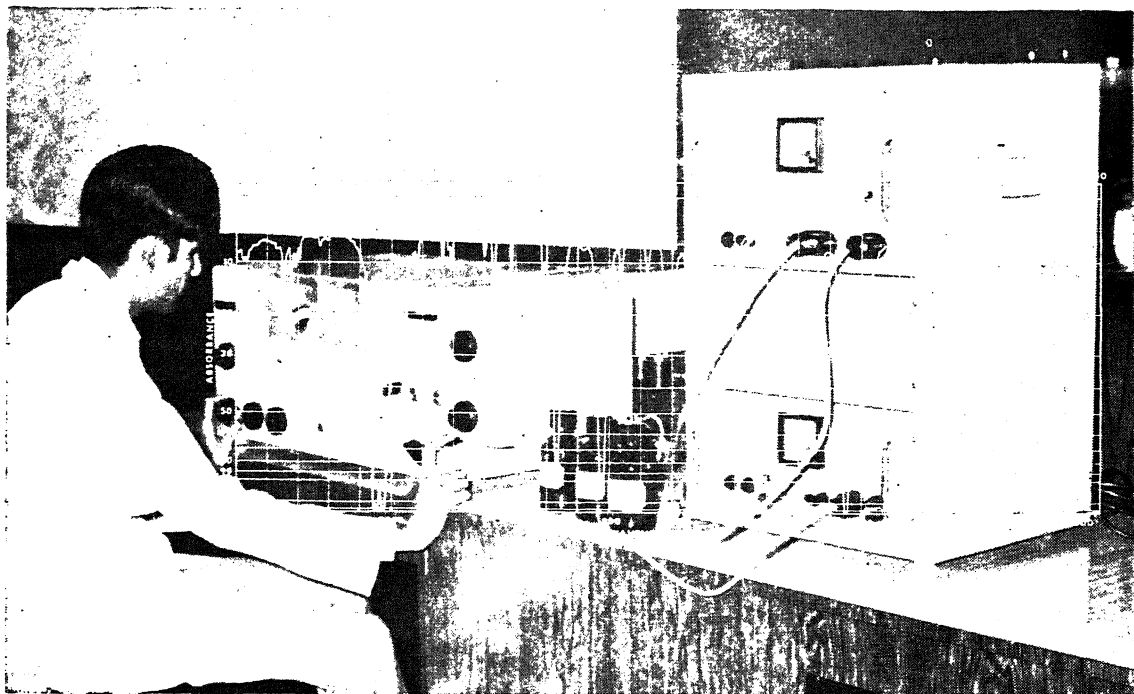
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## ABSTRACT

It has been shown that the solar daily, seasonal, latitudinal and solar cycle variations of the occurrence of counter equatorial electrojet is consistent with the idea that these events are caused by the superimposition of the lunar geomagnetic field wave over the solar wave to produce the observed daily variation of the geomagnetic H field at any place. The mechanisms for the amplification of the lunar tidal oscillation on a particular day are still to be sought.

**B**ARTELS and Johnston (1940) were the first to point out large deformations and decreases in the geomagnetic H field at Huancayo during the daytime hours of a magnetically quiet day. They suggested that on these days, the lunar daily variation is magnified so many times, of its expected value that it may even be greater than solar daily variation on the same day. Onwumechilli (1963, 1964) found the existence of such big lunar-tide days at Ibadan, even on such days on which the phase of lunar wave was completely reversed. This phenomenon of the decrease of H field during the daytime at an equatorial station, now called 'counter-electrojet' has been observed at Addis Ababa (Gouin and Mayaud, 1967), Zaria (Hutton and Oyinloye, 1970), Kodaikanal (Rastogi, 1973) and other stations near the magnetic equator (Rastogi, 1974a).

Gouin and Mayaud (1967) suggested that there is no connection between the occurrence of counter-electrojet and the moon as the phenomenon has been observed at all times of the lunar day. Similar opinion was expressed by Hutton and Oyinloye (1970) even though they found that counter-electrojet occurs most frequently around 03 and 15 lunar hours. Onwumechilli and Akasofu (1972) showed that there are cases when the time of counter-electrojets at Huancayo were in complete anti-phase with the L field, and considered it as an evidence against association of all cases of  $S_q(H)$  depression with the moon. Sastri and Jayakar (1972) found that major depressions of the H field at Trivandrum occurred most frequently around lunar age 00 and 12 hr, and were practically absent around 06 and 18 hr lunar ages.

Rastogi (1973) suggested that the observed solar and lunar times of maximum occurrence of afternoon counter-electrojet events are due to the superposition of the L field on the average S field. On the same model, he predicted that the morning counter-electrojet events should be most frequent around lunar ages 06 and 18 hr. This has been confirmed later from the data of Huancayo, where the morning counter-electrojet was found to be most frequent around lunar ages 05 and 17 hr (Rastogi, 1974b).

Recently, Rastogi (1974a) has published the results of a detailed study of the daily, seasonal and solar cycle variations of the counter-electrojet at a number of equatorial stations. Some of the major highlights of the above study revealed that : (i) the counter-electrojet at equatorial stations occurs around 0700 and 1600 LT, (ii) the evening events are twice as frequent as the morning events, (iii) out of all stations studied, the events are most frequent at Kodaikanal and least frequent at Huancayo, (iv) the number of counter-electrojet events are inversely proportional to the solar activity, (v) at Huancayo, the evening counter-electrojet events are more than seven times more frequent during summer (June) than during winter (December) months. For Kodaikanal the corresponding ratio is only about 2, and (vi) the latitudinal extent of the counter-electrojet events is confined within  $\pm 5^\circ$  dip latitude. It is the aim of this paper to investigate whether all these points are consistent with the idea of counter-electrojet being the result of the superposition of lunar wave on the normal solar daily wave. Each of the points is discussed in turn. The basic idea is that for any solar time (T), the value of the H field would be above or below the nighttime base value depending upon whether  $\Delta H$  due to average  $S_q$  field is larger or smaller than the decrease of H due to the lunar daily variation for that hour and corresponding to the lunar age of the day concerned. The relative frequency of the counter-electrojet with any geophysical parameter would be directly related to the variation of the ratio  $M_2H/\Delta H$  with the above parameter.

The solar cycle and the seasonal effects in the lunar daily variation of the H field at the equatorial stations have been described for Huancayo by Rastogi (1968) and for Kodaikanal by Trivedi and Rastogi (1969). The present paper has derived the data from these publications for studying the ratio  $M_2H/\Delta H$ .

First we examine the lunar effect on the occurrence frequency of the counter-electrojet event. In Fig. 1 are plotted the percentage frequency of occurrence of the afternoon counter-electrojet

events at number of stations in terms of the lunar age ( $\gamma$ ) and separately in terms of the lunar time ( $\tau$ ). Some of the results by earlier workers are also included in the diagram. It is very clearly shown here that the occurrence of the counter-electrojet is intimately associated with the lunar age or with the lunar time. The counter-electrojet is most frequent at lunar age around 02 hr, i.e., two days after new or full moon. In terms of lunar time, the counter-electrojet is most frequent at about 03 lunar hour, i.e., about 3 hours after the upper or lower transit of the moon.

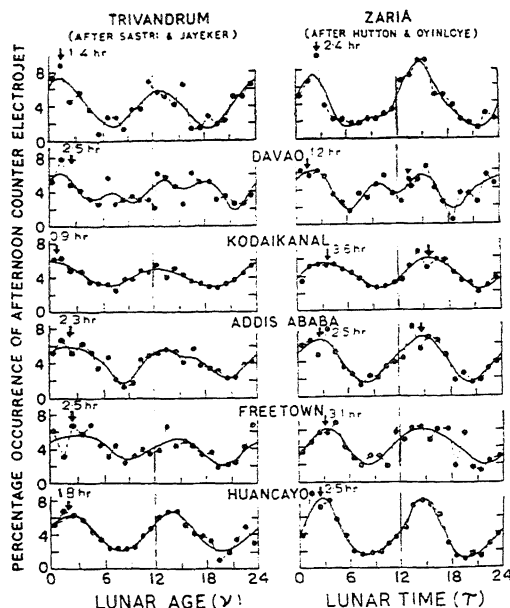


FIG. 1. The variation of the percentage occurrence frequency of the afternoon counter-electrojet events at number of equatorial stations as a function of lunar age and lunar time.

The lunar perturbations (due to semi-monthly wave) in  $\Delta H$  is known to be almost absent during the night hours and is maximum around midday (Rastogi and Trivedi, 1970). The lunar daily wave, however, has largest deviation at different solar time depending on the lunar age on that day. In Fig. 2 are shown the solar daily variation of  $H$  at Huancayo averaged over all quiet days of D-months in 1951-55. The deviation of  $H$  from this average curve is also shown for days with definite lunar ages; these curves show the lunar perturbation in terms of solar time. The largest deviation is seen on lunar ages 21 and 09 hr but the minimum occurs around 1000-1100 LT when  $\Delta H$  due to  $S_q(H)$  is comparatively much larger and so the lunar perturbations would not have any

apparent effect. On lunar ages 03 and 15 lunar hr large (about 10%) depression occurs around 1,600 LT when  $\Delta H$  due to  $S_q$  variation is less than 10%, and thus one may expect a negative value of  $\Delta H$  on those days due to combined effects of the moon and the sun. Thus the counter-electrojets in the evening hours are expected to be on days with lunar age around 03 or 15 hr. Similarly at the morning hours, large deviation in lunar wave comparable to  $S_q H$  at the same time, is expected on lunar ages around 18 and 06 hr. A more detailed picture can be obtained by studying the variation of the ratio  $(M_2/\Delta)H$  as a function of solar time. In Fig. 3, it is seen that although the maximum amplitude of lunar  $M_2(H)$  is about ten times smaller than the solar  $\Delta H$ , during the midday hours, the ratio  $(M_2/\Delta)H$  has two maxima in the morning and in the afternoon hours. The ratio is only about 0.1-0.2 during

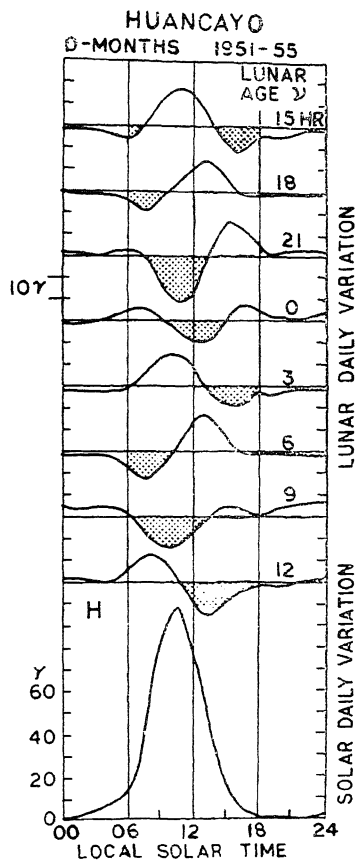


FIG. 2. Average solar daily variation of  $\Delta H$  at Huancayo for D-months compared with the corresponding lunar daily variations on days of definite lunar ages.

midday but is about 0.3–0.8 during 0700 LT or 1600–1700 LT. These two times are approximately the times when the counter-electrojets are most frequent.

should be most frequent during D-months while at Kodaikanal seasonal variation ought to be very feeble, which is again consistent with the observed fact.

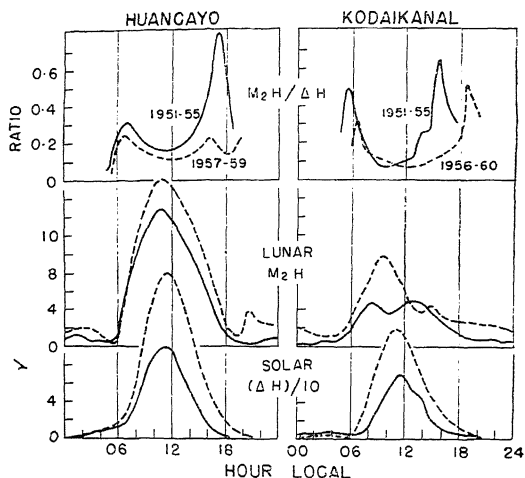


FIG. 3. The variation with the time of the day of the change of H field ( $\Delta H$ ), lunar semi-monthly tide in H ( $M_2H$ ) and the ratio  $M_2H/\Delta H$  for equatorial stations Huancayo and Kodaikanal for the year of low and high solar activity.

It is seen that on the average the ratio ( $M_2H/\Delta H$ ) is larger in the evening than in the morning hours suggesting greater probability of counter-electrojet in the evening than in the morning, which is consistent with the observed facts.

Examining the curves for the years of low and high solar activity, one finds that both  $M_2H$  as well as  $\Delta H$  are larger during years of high solar activity but the ratio  $M_2H/\Delta H$  is much smaller in the high than in the low sunspot years. This would suggest that the counter-electrojets should be less frequent during years of high sunspot number, which is again consistent with the observations.

It was also shown by Rastogi (1974a) that the number of afternoon counter-electrojet events at Huancayo is about 7 times more frequent in summer (D-months) than in winter (J-months); at Kodaikanal, the ratio is about 2 while at other stations the occurrence is practically independent of season. In Fig. 4 are shown the ratio  $M_2H/\Delta H$  at Huancayo and Kodaikanal as a function of local time for different seasons. It is seen that at Kodaikanal, the ratio is practically independent of season, but at Huancayo the maximum value of the ratio is about 1.8 in D-months and only 0.3 in J-months. Thus the counter-electrojet events at Huancayo

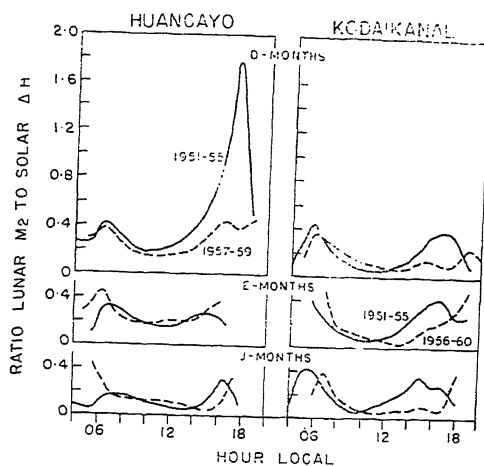


FIG. 4. The solar daily variation of the ratio of the amplitude of lunar semi-monthly tide in H ( $M_2H$ ) to the change in H from the mean nighttime level at each season of low and high sunspot years for Huancayo and Kodaikanal.

The diurnal, as well as seasonal variations of the occurrence of the counter-electrojet events at low-latitudes is consistent with the idea that the observed value of the H field is a combined effect of the solar variation and the lunar wave. There may be, of course, large deviations in this idea for individual cases of the counter-electrojet events.

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## MEMORY SWITCHING OF THIN CdS FILMS

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## ABSTRACT

Memory switching in thin CdS films has been studied and various switching parameters evaluated. The switching behaviour is attributed to the filling and emptying of barrier-surrounded traps whose existence is well defined.

## INTRODUCTION

**E**XTENSIVE studies have been made on memory switching of wide band-gap polycrystalline<sup>1,2</sup>, amorphous semiconductor materials<sup>3,4</sup>, various thin film heterojunctions<sup>5-7</sup> and other devices<sup>8,9</sup>. Memory in such a system has been attributed to a non-electronic process of phase transformation<sup>10</sup> or thermal diffusion of electrode metal<sup>11</sup>. However, these processes appear to be incapable of giving satisfactory explanation to the observed memory switching behaviour of the system. The present investigation has been made with a view to ascertaining the mechanism responsible for the observed behaviour in thin CdS films sandwiched between aluminium electrodes.

## EXPERIMENT

Thin films of CdS sandwiched between two aluminium electrodes were fabricated by vacuum evaporation technique on thoroughly cleaned glass slides by suitable masking arrangement. After each deposition, pellets of CdS were made before evaporation in order to avoid spurring of the material. The thickness of the film was monitored by the current through the heating filament and time of evaporation of CdS. Electrical contacts were made by pressure contact.

## RESULTS

The study of current voltage characteristic of the system exhibited two irreversible stages of conductivity. Initially a very low conductivity is observed and this is termed as virgin stage. This stage transforms irreversibly to a higher conductivity stage termed as post-breakdown stage, when the voltage across the sandwich exceeds a threshold value of about 7 to 10 volts. This stage exhibits memory switching between its two substrates of conductivity, viz., low and high conductivity states. An explanation to different breakdown potentials for transition has been given in terms of different effective ionized donor densities in the two states. This difficulty can be easily solved, if we know the contact field at breakdown. The contact field is

calculated to be of the order of  $10^6$  V/cm by using the well known expression<sup>12</sup>.

$$E = \left[ \frac{2qN(V + V_b)}{\epsilon\epsilon_0} \right]^{\frac{1}{2}}$$

where  $E$  is the contact field,  $V_b$  is the diffusion potential,  $q$  is the charge,  $\epsilon$  is the dielectric constant and  $\epsilon_0$  is the permittivity of the free space.  $V$  is the applied voltage and  $N$  is the ionized donor density.

Further, a detailed study of the conduction process of the system revealed that the conduction mechanism in both the stages of conductivity is due to field-induced generation of carriers followed by impact ionization.

The variation of current with voltage at different temperatures has been studied under steady-state conditions. The current decay after thermal stimulation gives the information about the energy and capture cross-section in the polycrystalline thin films. The current is observed to be time dependent and it assumes a steady state value after some time. All, but the shallowest traps are filled at room temperature when a fixed biasing potential is applied across the specimen. The number of majority carriers, i.e., ionized donor density, increases disturbing the equilibrium due to increase in the current. The trapping of excess carriers then takes place predominantly by shallow traps due to elevated temperatures. The decay of current, then starts taking place and after some time an appreciable fall is observed. Based on the above analysis of the current decay, two trap levels at 0.06 eV and 0.08 eV with respective capture cross-section  $4 \times 10^{-24}/\text{cm}^3$  and  $0.04 \times 10^{-24}/\text{cm}^3$  have been evaluated.

## CAPACITANCE STUDY

The study of the space charge capacitance of the diodes exhibited the existence of Schottky type space-charge capacitance due to thin aluminium oxide layer between bottom aluminium electrodes and cadmium sulphide. Further the ionized donor density in the pre-breakdown state is  $8.1 \times 10^{17}/\text{cm}^3$ .

$\text{cm}^2$  and in two reversible states are  $8.8 \times 10^{17}/\text{cm}^3$  and  $18.8 \times 10^{17}/\text{cm}^3$  respectively<sup>13</sup>. It is thus obvious from these data that the effective ionized donor densities in the pre-breakdown and low conductivity states are almost the same and show a marked variation from this value in the high conductivity stage.

The temperature variation of capacitance at low fixed bias voltage is shown in Fig. 1. This figure

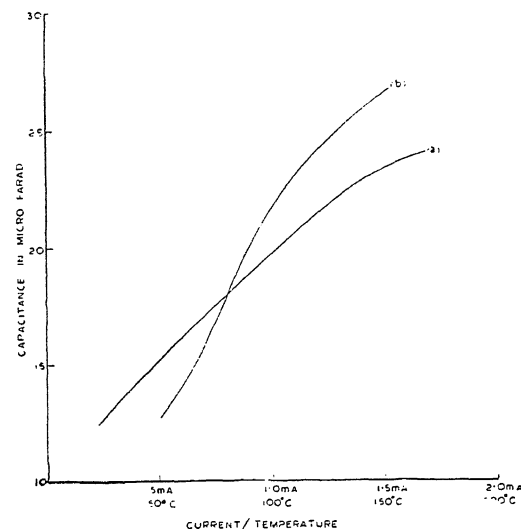


FIG. 1. Variation of capacitance with (a) current and (b) Temperature (at 2 volts).

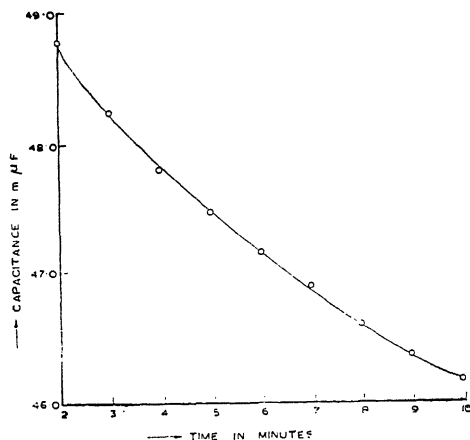


FIG. 2. The variation of capacitance with time (at 2 volts).

also shows the variation of capacitance with current at a constant voltage, current being changed by increasing the temperature. It is evident from this, that the capacitance and current

increase with temperature. The capacitance will increase only if trap ionization is the cause for increased current. Thus, it may be concluded that the major increase of the current with rising temperature is due to impact ionization of traps.

The capacitance of the system was also found to change its value with time immediately after a low bias was applied as shown in Fig. 2 which attains a steady value after about 25 minutes. The capacitance at fixed bias increases with time in the forward bias and decreases in the reverse bias operation. Furthermore this fall in capacitance was not observed when the sandwich was in the high conductivity state. It may, therefore, be concluded that the origin for both the phenomena, *i.e.*, change in capacitance and current with temperature are associated with ionization of slow traps.

### DISCUSSION

The inferences drawn from these experiments are used as a probe to explain the mechanism responsible for the observed memory switching behaviour of the system. The switching behaviour is attributed to either electrode metal diffusion, phase transformation or filling and emptying of barrier surrounded traps. The former alternatives, *i.e.*, electrode metal diffusion and phase transformation, do not yield satisfactory explanation to the change of donor density by a factor of two in different states of post-breakdown stage. The rapidity of the switching behaviour of the system also does not favour the idea of phase transformation. Cadmium sulphide in contact with aluminium oxide forms a depletion type of contact as shown in Fig. 3. Cadmium sulphide is an N-type semi-

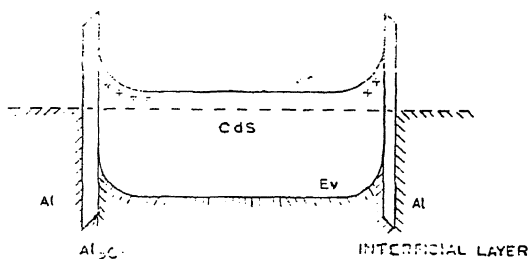


FIG. 3. Energy Band diagram of Al-Al<sub>2</sub>O<sub>3</sub>-CdS Film.

conductor and hence electrons are depleted from aluminium oxide cadmium sulphide interface when positive voltage is applied to the top electrode of the sandwich. For larger voltage, impact ionization dominates field ionization. Thus by Avalanche process, trapping centres which are in excess near aluminium oxide-cadmium sulphide interface are emptied out. This causes an increase in the effective

ionized donor density and hence an increase in the field for the same voltage is achieved. The increase in the field will further enhance impact ionization and donor density further increases and the entire process becomes cumulative and instantaneous till all the traps are ionized and switching takes place. The transition from high to low conductivity state can be explained on similar lines. In this case negative bias is applied to the upper electrode (i.e., the direction of the bias is reversed). The reverse bias increases the width of the depletion region near cadmium sulphide aluminium interface and for large applied voltage, impact ionization supercedes field ionization. Thus warm electrons are generated near cadmium sulphide aluminium interface which are trapped by barrier surrounded trapping centres near oxide semiconductor interface. This reduces the effective ionized donor density thereby resulting in a decrease of conductivity.

#### CONCLUSION

It is thus concluded that the memory switching in the system considered is due to filling and emptying of barrier-surrounded traps, which change its occupancy and hence the conductivity at threshold voltage when switching takes place.

#### ACKNOWLEDGEMENT

The authors wish to record their sincere gratitude to Dr. S. S. Banerjee, UGC, Professor, for his encouragement throughout the course of the investigation. Thanks are also due to CSIR, India, for supporting the project.

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### MAGNETIC PROPERTIES OF COPPER (II) COMPLEXES OF SCHIFF BASES DERIVED FROM PYRROLE-2-ALDEHYDE AND ISOPROPANOLAMINE/2-AMINO-2-METHYLPROPANOL

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#### ABSTRACT

The synthesis of new copper(II) complexes of Schiff bases derived from pyrrole-2-aldehyde and isopropanolamine or 2-amino-2-methylpropanol is described. The Schiff bases coordinate through O, N and N as tridentate dibasic ligands. The complexes are characterised by magnetic susceptibility (84–294° K), infrared and electronic spectral studies. The magnetic moment of the copper (II) complex of pyrrole-isopropanolamine increases as the temperature is lowered indicating ferromagnetic nature of the complex while that of the copper(II) complex of pyrrole-2-amino-2-methylpropanol decreases with lowering of temperature indicating antiferromagnetic nature of the complex. The difference in magnetic properties has been attributed to the presence of steric hindrance arising out of two methyl groups in the latter complex.

#### INTRODUCTION

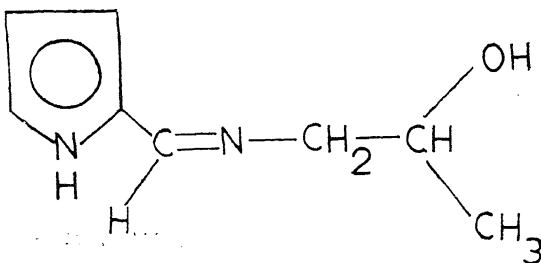
TRIDENTATE dibasic ligands yield metal complexes which frequently exhibit novel structural and magnetic properties<sup>1-3</sup>. The dibasic character of these ligands force the metal (II) ions to dimerise or polymerise leading to metal complexes with unusual magnetic properties. The size of the chelate rings influence the magnetic properties of the copper (II) complexes<sup>4</sup>. We now report the effect of steric hindrance on the magnetic behaviour

of the copper (II) complexes of Schiff bases. We have prepared new copper (II) complexes with the tridentate dibasic ONN donor ligands I and II and studied the magnetic properties of the complexes from 84 to 294° K.

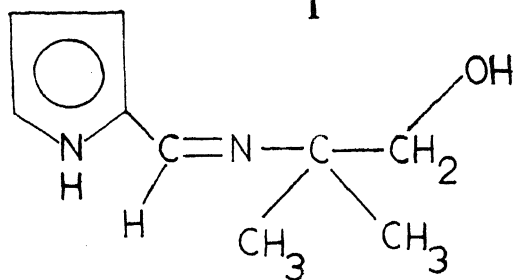
#### EXPERIMENTAL

Pyrrole-2-aldehyde (0.02 M) was dissolved in 40 ml ethanol and isopropanolamine (0.02 M) was mixed with 10 ml ethanol. These solutions were

mixed and the mixture was refluxed on a water bath for 20 min. to give a yellow solution of the Schiff base. An aqueous solution of copper (II) nitrate trihydrate (0.01 M in 20 ml) was added slowly to the Schiff base solution with stirring followed by an aqueous solution of sodium carbonate (0.01 M in 20 ml). The mixture was stirred magnetically at 60°C for one hr and then cooled to room temperature. The separated green precipitates were suction filtered washed with ethanol and dried under vacuum. It was recrystallised from benzene and dried under vacuum (Yield 50%).



I



II

*Cu*(pyrrole-2-amino-2-methylpropanol) was prepared by following a similar procedure as described above. The complex was recrystallised from chloroform. (Yield 50%).

The microanalyses were done at the University of Bombay. The analytical data are given in Table I. The magnetic susceptibility measurements

were done by the Gouy method. Diamagnetic corrections of the metal and ligand atoms were calculated using a standard source<sup>5</sup>. Electronic spectra were recorded in chloroform with a Beckman DK2 recording spectrophotometer using 10 mm matched quartz cells. IR spectra were recorded in KBr pellets on a Perkin Elmer Model 21 instrument. The molecular weights were determined in chloroform at 37°C using a Hewlett-Packard Mechrolab Model 301A vapour pressure osmometer calibrated with benzil.

## RESULTS AND DISCUSSION

The infrared spectra of the complexes do not exhibit the  $\nu(\text{OH})$  stretching vibration indicating the ligand coordination, dibasic character of the ligands and the absence of coordinated water in the complexes. The osmometric molecular weight measurements in chloroform indicate that the copper (II) complex of I is tetrameric (Found : 840, Calcd. 854), and of II is dimeric (Found : 410, Calcd. 455). Apparently the steric factor associated with the presence of two methyl groups in the ligand II is preventing the formation of tetrameric copper (II) complex. The electronic spectra in chloroform of copper (II) complexes of I and II exhibit a broad absorption band at 15950 and 17030  $\text{cm}^{-1}$  respectively. This difference in band position may be attributed to the presence of different structures in these complexes<sup>6,7</sup>.

The magnetic susceptibilities and magnetic moments of the complexes at several temperatures are presented in Table II. The magnetic moments of the copper (II) complex of I increase as the temperature is lowered. On the other hand, the magnetic moments of the copper (II) complex of II decrease with lowering of temperature. Approximating the magnetic properties using the Bleaney and Bowers equation<sup>8</sup> gave positive  $J$  value ( $J = +35 \text{ cm}^{-1}$ ) for the copper (II) complex of I and negative  $J$  value ( $J = -133 \text{ cm}^{-1}$ ) for the copper (II) complex of II. Thus the magnetic data indicate that the copper (II) complexes of I and II are ferromagnetic and antiferromagnetic,

TABLE I

Analytical and electronic spectral data of copper (II) complexes<sup>a</sup>

| Complex                               | Stoichiometry                                 | %C                              | %H           | %N           | $\nu$ max ( $\epsilon$ ) |
|---------------------------------------|---|---------------------------------|--------------|--------------|--------------------------|
| Cu (pyrrole-isopropanol-amine)        | $\text{CuC}_8\text{H}_{10}\text{N}_2\text{O}$ | Calcd. : 44.95<br>Found : 44.60 | 4.68<br>4.70 | 13.1<br>13.0 | 16950<br>(140*)          |
| Cu (pyrrole-2-amino-2-methylpropanol) | $\text{CuC}_9\text{H}_{12}\text{N}_2\text{O}$ | Calcd. : 47.47<br>Found : 47.60 | 5.3<br>6.0   | 12.3<br>12.1 | 17030<br>(115*)          |

<sup>a</sup> Abbreviation : pyrrole=pyrrole-2-aldehyde.

\* litre mole<sup>-1</sup> cm<sup>-1</sup>

TABLE II

Magnetic susceptibilities and magnetic moments of copper (II) Schiff base complexes<sup>a, b</sup>

| Cu (pyrrole-isopropanolamine) |  |                            |                                    | Cu (pyrrole-2-amino-2-methyl-propanol) |  |                            |                                    |
|-------------------------------|--|----------------------------|------------------------------------|--|--|----------------------------|------------------------------------|
| Temp.<br>(°K)                 | $\chi_{\text{M}}^{\text{corr}}$<br>( $10^{-6}$ cgs unit) | $\mu_{\text{eff}}$<br>(BM) | $J^{\text{e}}$<br>$\text{cm}^{-6}$ | Temp.<br>(°K)                          | $\chi_{\text{M}}^{\text{corr}}$<br>( $10^{-6}$ cgs unit) | $\mu_{\text{eff}}$<br>(BM) | $J^{\text{e}}$<br>$\text{cm}^{-1}$ |
| 293                           | 1371   | 1.80                       |                                    | 294                                    | 1059   | 1.58                       |                                    |
| 215                           | 1946   | 1.83                       |                                    | 215                                    | 1295   | 1.50                       |                                    |
| 161                           | 2646   | 1.85                       | +35                                | 159                                    | 1553   | 1.41                       | -133                               |
| 128                           | 3388   | 1.87                       |                                    | 126                                    | 1694   | 1.31                       |                                    |
| 101                           | 4310   | 1.87                       |                                    | 105                                    | 1764   | 1.22                       |                                    |
| 84                            | 5283   | 1.89                       |                                    | 84                                     | 1812   | 1.11                       |                                    |

<sup>a</sup> Magnetic moment was calculated using the Curie equation:

$$\mu_{\text{eff}} = 2.84 (\chi_{\text{M}}^{\text{corr}} T)^{\frac{1}{2}} \text{ BM.}$$

<sup>b</sup> T.I.P. =  $50 \times 10^{-6}$  cgs units.<sup>c</sup>  $g = 2.05$  was used.

respectively<sup>1,4,9,10</sup>. This difference in magnetic behaviour in these two complexes may be related to the presence of different structures in these complexes—One a tetramer and the other, a dimer. As the ligands I and II both form five membered chelate rings around copper (II) in aminoalcohol part of the molecules, we believe this significant difference in magnetic behaviour is due to the steric hindrance of the two methyl groups which prevent the formation of tetramer in copper (II) complex of II. On the basis of X-ray structure determination of known copper (II) complexes of tridentate dibasic Schiff bases with comparable magnetic properties, we propose a square planar structure for the dimeric, antiferromagnetic copper (II) complex of II and a distorted trigonal bipyramidal arrangement for the tetrameric, ferromagnetic copper (II) complex of I<sup>167</sup>.

It is interesting to note that the copper (II) complex of pyrrole-2-aldehyde-propanolamine is dimeric with square planar arrangement around each copper (II) and the complex is involved in the antiferromagnetic spin-spin coupling<sup>6</sup>. This ligand forms a six membered chelate ring around copper (II) in aminoalcohol part of the molecule. When the size of the chelate ring is five membered in the copper (II) complex of the ligand I one gets a ferromagnetic copper (II) compound. We do not

see this chelate ring effect in the copper (II) complex of the ligand II due to the steric hindrance as described earlier.

The authors are indebted to the Council of Scientific and Industrial Research for financial support.

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## PHOTOSYNTHETIC CARBOXYLASES IN WILD AND CULTIVATED WHEATS

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IN recent years physiological aspects such as photosynthesis rates, nitrate reductase activity, chlorophyll and nitrogen content have been examined in primitive and cultivated wheats (Evans and Dunstone, 1970; Khan and Tsunoda, 1970, Sinha and Khanna, 1972. Sinha *et al.*, 1974). These studies reveal that the primitive wheats are characterized by higher photosynthesis rates (Evans and Dunstone, 1970, Khan and Tsunoda, 1970). We report the variation in the activities of RuDP carboxylase (Ribulose 1-5 diphosphate carboxylase) and PEP carboxylase (phosphoenol pyruvate carboxylase) in diploid, tetraploid and hexaploid wheats during growth and development in flag leaf and ear parts. These were the same genotypes which we examined for variation in photophosphorylation (Sinha and Khanna, 1972).

Seeds of a diploid *Triticum monococcum* L., a tetraploid *T. durum* Desf. and a hexaploid *T. aestivum* Villcv. Kalyansona were sown in pots and plants were raised as described earlier (Sinha and Khanna, 1972). The activities of RuDP carboxylase and PEP carboxylase were determined after the emergence of flag leaf. These enzymes were assayed according to the methods described by Bjorkman and Gauhl (1969) and Khanna and Sinha (1972). Photosynthetic carboxylase activity has been shown to be positively correlated with photosynthesis rate by Neals, Treharne and Wareing (1971) and Nagy *et al.* (1972). Results are expressed on fresh weight of leaves, awns, and glumes.

RuDP carboxylase activity in the flag leaf of diploid and tetraploid wheat was more than that in hexaploid before anthesis occurred (Table I). At anthesis, the activity of this enzyme was enhanced by 50% in tetraploid and about three times in hexaploid wheat. Earlier results had shown enhanced CO<sub>2</sub> fixation and O<sub>2</sub> evolution at anthesis in Kalyansona, a hexaploid type (Sinha unpublished). When the grain development started the diploid still maintained the same enzyme activity but it declined in the other two genotypes as compared to the anthesis stage.

The activity of PEP carboxylase was very low as compared to RuDP carboxylase. There was decline in the activity of this enzyme following anthesis in tetraploid and hexaploid but was less affected in diploid.

TABLE I

*RuDP carboxylase and PEP carboxylase activities in the flag leaves of diploid, tetraploid and hexaploid wheats following ear emergence*

| Genotype                    | $\mu$ moles CO <sub>2</sub> fixed g <sup>-1</sup> f.w. min <sup>-1</sup> |      |          |      |               |      |
|-----------------------------|--|------|----------|------|---------------|------|
|                             | Preanthesis  |      | Anthesis |      | Post anthesis |      |
|                             | RuDP   | PEP  | RuDP     | PEP  | RuDP          | PEP  |
| Diploid                     | 5.0  | 0.70 | 4.7      | 0.43 | 5.8           | 0.62 |
| Tetraploid                  | 5.0  | 0.97 | 7.5      | 0.85 | 6.8           | 0.64 |
| Hexaploid<br>Cv. Kalyansona | 3.1  | 0.87 | 9.5      | 0.33 | 5.5           | 0.54 |

TABLE II

*RuDP carboxylase activity in the awns and glumes of diploid, tetraploid and hexaploid wheats*

| Genotype                    | $\mu$ moles CO <sub>2</sub> fixed g <sup>-1</sup> f.w. min <sup>-1</sup> |        |          |        |               |        |
|-----------------------------|--|--------|----------|--------|---------------|--------|
|                             | Preanthesis  |        | Anthesis |        | Post anthesis |        |
|                             | Awns   | Glumes | Awns     | Glumes | Awns          | Glumes |
| Diploid                     | 3.7  | 1.1    | 3.6      | 1.4    | 4.6           | 1.7    |
| Tetraploid                  | 3.9  | 1.1    | 6.7      | 3.8    | 6.5           | 8.0    |
| Hexaploid<br>Cv. Kalyansona | 2.7  | 1.4    | 4.5      | 2.2    | 3.4           | 2.4    |

Awns of wheat and barley are known to contribute photosynthetically towards grain yield (Yoshida, 1972) RuDP carboxylase activity of awns was higher in diploid and tetraploid at preanthesis (Table II). At anthesis the enzyme activity in awns increased in tetraploid and hexaploid but was unaffected in diploid. In glumes which are even more closer to grains the activity of RuDP carboxylase was almost the same in all the types before anthesis (Table II). At anthesis maximum stimulation in the activity of this enzyme occurred in the tetraploid, although slight increase was observed in diploid and hexaploid types. Very low PEP (0.09–0.64  $\mu$  moles CO<sub>2</sub> fixed g<sup>-1</sup> fw min<sup>-1</sup>) carboxylase activity was observed in the awns and glumes at all the three stages.

It is known that the ear size, grain number and grain weight increases from diploid to hexaploid leading to greater 'sink' capacity (Evans and Dunstone, 1970). Earlier studies were confined to seedlings wherein higher photosynthesis rates were obtained in primitive types (Evans and Dunstone, 1970; Khan and Tsunoda, 1970). The creation of 'sink' seems to have a profound effect on the photosynthetic potential of wheat leaves and other photosynthetically active parts. It would be seen from Table I that the flag leaf of hexaploid had poor rates before anthesis but reached maximum at anthesis. A similar observation was made in respect of photophosphorylation (Sinha and Khanna, 1972). However, the glumes and awns of tetraploid type had higher amount of RuDP carboxylase. Our recent studies indicate a large amount of photosynthate contribution by awns and glumes to the developing grains of wheat (Khanna and Sinha, 1973).

The present study indicates that the primitive wheats do not have higher photosynthesis rates at all stages of development as compared to the advanced types. The photosynthetic potential

changes with the stage of development and the availability of 'sink'. Thus comparisons of physiological traits may have only a limited significance in determining evolutionary relationships.

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## LETTERS TO THE EDITOR

### EFFECT OF ANNEALING ON THE CATALYTIC ACTIVITY OF COPPER\*

BHAKTA AND TAYLOR<sup>1</sup> have shown that gold catalyst, on annealing *in vacuo* in the temperature range of 200° to 400° C followed by quenching at liquid air temperature, acquired excess activity (towards the dehydrogenation of formic acid) which decayed out at a lower anneal temperature. The present investigation was undertaken to ascertain whether copper would also show a similar effect upon annealing in air for one hour at 250° to 550° C followed by either (a) quenching in water at 25° C, or (b) slow cooling in a furnace. The results revealed some unexpected interesting features. Square pieces (3.0 cm × 3.0 cm) of copper foils (99.9% purity, B.D.H.) from separate lots I, II and III, of mean thickness 0.10 mm, 0.12 mm and 0.13 mm, respectively, were used; the gm. equiv. of hydrogen peroxide (0.25 N) decomposed per unit surface area of the foil during the initial 10 min. of the reaction at 69.5° C at a stirrer speed of 200 rpm was taken as a measure of the activity,  $a$ , of the foil.

As the activity of the unannealed foils were found to be nearly proportional to the reciprocals of their thickness, mapping of the annealing data of the three foils on a single scale based on  $a_0$  ( $1.58 \times 10^{-4}$  gm. equiv. cm.<sup>-2</sup>, the activity of the unannealed foil I) was possible. (Fig. 1).

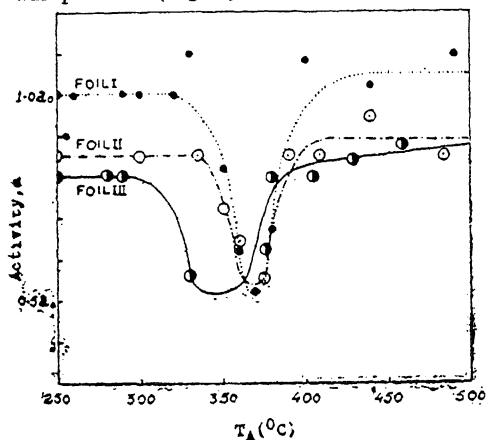


FIG. 1

This investigation has revealed that

A. the activities of the foils I, II and III.

- (i) remained, apparently, constant at their respective unannealed values (i.e.,  $a_0$ ,  $0.85 a_0$ ,  $0.80 a_0$ , respectively) upto  $T_A \sim 300^\circ \text{C}$ .

- (ii) attained the same minimum residual activity ( $0.5 a_0$  approximately) in the same  $T_A$  range of  $330^\circ \text{C}$  to  $380^\circ \text{C}$ .
- (iii) showed similar trend of activity increase (following the minima) to reach once again values nearly equal to their respective unannealed ones, at about the same temperature range ( $380^\circ \text{C}$  to  $420^\circ \text{C}$ ) and remained practically constant at  $T_A > 420^\circ \text{C}$ .
- (iv) showed the same pattern of behaviour, viz., (i), (ii) and (iii) when the foils were furnace-cooled (instead of quenched) after annealing at  $T_A$  range  $250^\circ \text{C}$  to  $550^\circ \text{C}$ .

and

B. the Vickers microhardness of foil II remained unchanged upto  $T_A \sim 420^\circ \text{C}$ , thereafter it decreased to a final constant value (70% of the initial value) at  $T_A \sim 480^\circ \text{C}$ .

In passing, it can be noted that the onset of 'softening' of a foil began at about  $420^\circ \text{C}$  at which it attained the maximum activity. At the present moment it is not possible to say if this is a mere coincidence.

The activity decay in the  $T_A$  range  $300^\circ \text{C}$  to  $380^\circ \text{C}$  follows a pattern similar to that observed by Eley and MacMahon<sup>2</sup> and Uhara *et al.*<sup>3</sup>. The present investigation succeeds in clearly delineating the complete picture of the changes in the activity of the foils in the  $T_A$  range of  $250^\circ \text{C}$  to  $550^\circ \text{C}$ .

It seems likely that the free surface of the copper foil is itself active accounting for the unannealed activity ( $0.5 a_0$ ). The activity of the foil in excess of  $0.5 a_0$  may be attributed to the surface terminations of dislocations in accordance with the views of Cratty and Granato<sup>4</sup>; the annealing characteristics of its hardness are also consistent with these views. These dislocations are, perhaps, introduced in the foils when they were cold-rolled (during manufacture); as the thinness of a foil is, presumably, proportional to the amount of cold-work performed and, consequently, to the number of dislocations introduced, the reason for the observed dependence of the activity of a foil on the reciprocal of its thickness, becomes clear.

The rise in the activity following the minimum is, however, not easy to interpret. There is, undoubtedly, visible roughening of the surface of the foil after annealing at  $T_A > 380^\circ \text{C}$ . But this alone is not sufficient to account for the observed increase in the activity; nor can this increase be attributed



to the streams caused by quenching. As the quenched foil did not behave differently from the furnace-cooled one. As every foil was first immersed in 1*N*  $H_2SO_4$  for 3 to 4 min. (until it regained its shine) before it was used as a catalyst, the probability that the oxide film on its surface might be responsible for the increase in activity is small. Incidentally, it is worth noting that silver catalyst, too, exhibited similar increase in activity<sup>2</sup> when hydrogen peroxide decomposition and ethanol oxidation (and not formic acid dehydrogenation) were the probe reactions. This observation may be of significance to the understanding of the mechanism of metal catalysed reactions.

Department of Chemistry, K. Sankar,  
Dharmpe College of Arts and M. A. Bhaskar,  
Science,  
Punjab, Goa, August 27, 1974.

\* This communication is based on the dissertation submitted by K. S. to the University of Bombay for the M.Sc. degree.

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### ELECTRONIC SPECTRAL AND THERMAL STUDIES OF Ce(III), Pr(III) AND Nd(III) PICRAMATES

SOME inner transition metal picramates, viz. lanthanum, cerium, praseodymium, neodymium, samarium, gadolinium, dysprosium and holmium picramates, were prepared recently by us and their stoichiometry established by elemental analysis, amperometric, conductometric and potentiometric

analysis<sup>1</sup>. We also suggested  $-H_2N \rightarrow Ln$  coordination ( $Ln$  = rare earth ion) in metal picramates on the basis of i.r. spectral studies. The present communication reports the electronic spectral and thermal studies of cerium, praseodymium and neodymium picramates. The nature of the complexes has also been studied by measurements of electrical conductance.

The conductance and electronic spectra of the complexes were made as reported in the earlier communication<sup>2</sup>.

The low molar conductivity of the complexes in dimethylformamide showed that these complexes are non-electrolytes. The spectral data of praseodymium and neodymium picramates in ethanol are summarised in Table I, and for comparison, data for aqueous salt solutions are also given.

The spectral behaviour of lanthanides is fundamentally different from that of *d*-block elements. The basic reasons for the differences lies in the fact that the electrons responsible for magnetic spectral properties of lanthanide ions of 4*f* electron and 4*f* orbitals are effectively shielded from interaction with external forces by the overlapping of 5*s*<sup>2</sup> and 5*p*<sup>6</sup> shells. The bands may be assigned to the transitions in which an *f* electron is excited to an outer *d*, *s* or *p* orbital<sup>3</sup> and the spectra have been assigned to 4*f*–5*d* transitions<sup>4</sup>. The electronic spectra of the cerium picramate show five bands appearing at 21735 (vw), 23250 (vw), 31755, 44455 and 47660  $cm^{-1}$ . The first three bands may be due to Laporte-allowed 4*f*–5*d* transitions.

It is evident from Table I that the shifts in the spectra of praseodymium and neodymium picramates are towards lower wave numbers relative to the aquo ions. There are small differences in frequencies or molar absorption except that the hypersensitivity transition  $^4I_{9/2} \rightarrow ^4G_{7/2}$  in the neodymium picramate which increases in intensity from  $\epsilon = 6.25$  in the salt to  $\epsilon = 17.50$  in the com-

TABLE I

Electronic spectra of praseodymium and neodymium nitrate and their corresponding complexes

|         | Transition                         | Ln(NO <sub>3</sub> ) <sub>3</sub><br>in water<br>( $cm^{-1}$ ) | $\epsilon$ salt | [Ln(C <sub>6</sub> H <sub>5</sub> ) <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub><br>(NH <sub>2</sub> O) <sub>2</sub> ·2H <sub>2</sub> O]<br>in ethanol ( $cm^{-1}$ ) | $\epsilon$ complex |                 |
|---------|------------------------------------|--|-----------------|--|--------------------|-----------------|
|         |                                    |  |                 |  | $\epsilon$ complex | $\epsilon$ salt |
| Pr      | $^2H_{1/2} \rightarrow ^1D_2$      | 17,000   | 1.90            | 16,860 (0.0083)*   | 2.10               | 1.36            |
|         | $\rightarrow ^3P_0$                | 20,750   | 3.00            | 20,760 (0.0024)*   | 3.55               | 1.18            |
|         | $\rightarrow ^3P_1$                | 21,320   | 4.20            | 21,140 (0.0085)*   | 11.76              | 2.80            |
|         | $\rightarrow ^3P_2$                | 22,470   | 9.55            | 22,430 (0.0017)*   | 10.89              | 2.09            |
| $^4I_9$ | $\rightarrow ^4F_{3/2}, ^2H_{9/2}$ | 12,500   | 6.42            | 12,450 (0.0040)*   | 11.90              | 1.85            |
|         | $\rightarrow ^4F_{7/2}, ^2S_{1/2}$ | 13,400   | 5.85            | 13,310 (0.0067)*   | 9.20               | 1.57            |
| Nd      | $\rightarrow ^4G_{5/2}, ^2G_{7/2}$ | 17,700   | 6.25            | 17,150 (0.0087)*   | 17.50              | 2.80            |
|         | $\rightarrow ^4G_{7/2}$            | 19,000   | 3.36            | 18,910 (0.0047)*   | 6.26               | 1.86            |
|         | $\rightarrow ^4G_{9/2}$            | 19,500   | 1.65            | 19,400 (0.0051)*   | 3.66               | 2.21            |

\* Values in parentheses show the nephelauxetic effect (1– $\beta$ ).

plex. Thus it is clear that 4f orbitals do not participate much in bonding; if they did, vibronic coupling would lead to marked changes in intensity, position, and sharpness of the  $f-f$  transitions. Four bands have been observed in the electronic spectra of praseodymium picramate which are due to transition from the ground level  $^3H_4$  to the excited J levels of 4f<sup>2</sup> configuration respectively. The red shift or nephelauxetic effect which is regarded as a measure of the covalency of the coordination bond was calculated for the rare earth complexes by Jørgensen *et al.*<sup>5</sup>, using the relation

$$1-\beta = (\bar{\nu}_{\text{aqu}} - \bar{\nu}_{\text{complex}}) / \bar{\nu}_{\text{aqu}}$$

(where  $\bar{\nu}$  is the wave number of absorption band of rare earth ion) and assuming that each J level of a 4f<sup>n</sup> configuration is linearly dependent on the radial integrals. On the basis of this assumption,  $\Delta\bar{\nu}/\bar{\nu}_{\text{aqu}}$  of all bands of the same rare earth complex should be similar. However, our earlier observations<sup>3</sup> as well as the present results (Table I) indicate that the  $\Delta\bar{\nu}/\bar{\nu}_{\text{aqu}}$  values for different J levels of a rare earth complex differ from each other considerably.

**Thermal Study.**—Thermogravimetric analysis of the samples was done by the method described by Agrawal *et al.*<sup>8</sup>. In the case of Ce picramate one water molecule is lost around 90°C and the second water molecule around 128°C. On the other hand, in the case of Pr and Nd picramates both the water molecules are lost around 130°C. Ce, Pr and Nd picramates decompose into the stable oxides (Ln<sub>2</sub>O<sub>3</sub>) at 450°, 650° and 700°C respectively; the increase in the thermal stability is perhaps due to a decrease in the O-Ln bond on account of lanthanide contraction.

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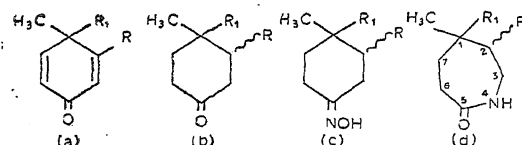
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# ε-CAPROLACTAMS FROM 4-ALKYL-, 4-DIHALO- AND 4-TRIHALO-ALKYL-2, 5-CYCLOHEXADIENONES

In our earlier publications<sup>1</sup> we have reported some of the interesting reactions of 4-alkyl-, 4-dihalo- and 4-trihalo-alkyl-2,5-cyclohexadienones. We report here the preparation of three caprolactam derivatives from these dienones. The latter were prepared from *p*-alkyl phenols by reaction with chloroform or carbon tetrachloride as described in literature and were catalytically reduced in alcohol solution with 5% Pd/charcoal to yield the corresponding cyclohexanone derivatives. The oximes of the latter, prepared as usual, when subjected to a Beckmann rearrangement in the presence of conc. sulphuric acid or PCl<sub>5</sub> afforded the lactams as colourless crystalline solids from benzene-pet. ether in yields ranging from 30–50% as in the case of III d. The rearrangement of the oxime IIc takes place only with PCl<sub>5</sub>. Sulphuric acid however gives a tarry mass.



I. R = H, R<sub>1</sub> = CHCl<sub>2</sub> :

(a) m.p. 54°<sup>2a</sup> ; (b) m.p. 47°<sup>2b</sup>  
(c) m.p. 132–34° ; (d) m.p. 152–54°.

II. R = H, R<sub>1</sub> = CCl<sub>3</sub> :

(a) m.p. 104°<sup>3a</sup> ; (b) m.p. 116°<sup>3b</sup> ;  
(c) m.p. 170–71° ; (d) m.p. 160–61°.

III. R = CH<sub>3</sub>; R<sub>1</sub> = CHCl<sub>2</sub> :

(a) m.p. 102°<sup>2a</sup> ; (b) m.p. 87° ;  
(c) m.p. 157° ; (d) m.p. 172–74°.

In the case of the oximes there is the possibility of syn- and anti-isomers being formed but in all cases only one product could be isolated. Similarly, in the case of compounds III b–III d, the possibility of *cis-trans* isomers also exists but here again only one compound could be obtained. From the spectral data collected by us it is not possible to assign any definite configuration to the methyl group in these compounds.

The i.r. spectrum (KBr) of a typical caprolactam (Id) showed bands at 3200, 1660 and 1300 cm<sup>-1</sup> corresponding to an amide (secondary) grouping.

The n.m.r. spectrum (CDCl<sub>3</sub>) of III d showed the methyl protons at C<sub>1</sub> as a singlet at 1.3 δ while a doublet around 0.9–1.1 δ for three protons was assigned to the methyl protons at C<sub>2</sub>. The complex multiplet at 1.5–3.9 δ for seven protons corresponded to the six methylene and one methine proton at C<sub>2</sub>. The singlet for one proton at 5.65 δ was

attributed to the proton of the dichloromethyl group at  $C_1$ . The NH proton was obtained as a broad singlet at 7.12  $\delta$ .

All the compounds gave satisfactory analysis for C, H and N.

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### SUBSTITUENT EFFECT OF THE $CF_3$ GROUP IN AROMATICS

THE effect of the  $CF_3$  group on the electron distribution of the phenyl part has been discussed by many workers<sup>1-6</sup>. Different mechanisms such as fluoride type no bond resonance, hyperconjugation and similar other mechanisms have been suggested to explain the strong electron withdrawing effect of the  $CF_3$  group.

Spectra of isomeric trifluorotoluidines  $CF_3C_6H_4NH_2$  have been studied in detail in solution as well as in the vapor phase. The solution spectra are shown in Fig. 1. On the basis of a large

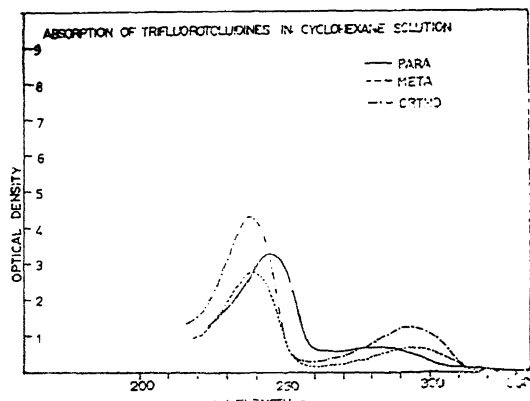


Fig. 1

amount of data on substituted anilines, Forbes and Leckie<sup>7</sup> have drawn some conclusions regarding the effect on the ultraviolet spectrum of aniline as a function of the nature of the substituent. Present results are discussed in the light of these as also general considerations of substituent effect.

The two bands generally observed in the spectra of substituted anilines in the region above 200  $m\mu$

are the usual B and C bands, the former being around 240  $m\mu$  and the latter around 295  $m\mu$ .

It has generally been accepted that the B band has a large contribution of intramolecular charge transfer state. The effect in para substituted anilines when the other substituent is an electron withdrawing, is a large bathochromic shift of the aniline B band and the C band remaining more or less in the same position. In some cases two bands merge giving only a broad single band. In the case of ortho and meta isomers, for acceptor substituents, quite often, two B bands are observed and the C band shows a large bathochromic shift.

In  $CF_3$  substituted anilines, since  $CF_3$  is an acceptor group, the spectral changes in general follow the above pattern, but there are some pertinent differences. First, the shift of the C band in meta and para isomers is much less than for other electron accepting substituents like formyl, acetyl with delocalised  $\pi$  orbitals. Secondly the B band does not show a splitting in ortho and meta isomers as in other cases. This shows that the electron withdrawal mechanism of  $CF_3$  group is different from the usual acceptors like formyl.

It is known that the spectral effects in case of unsaturated acceptors like formyl are principally due to  $\pi$  electron induction. Splitting of the B band in ortho and meta substitutions is due to the separation of the benzenoid B band and the aniline charge transfer band resulting from the pronounced lowering of the latter. The shift of the B band in para and of C in ortho and meta are also due to the some electronic effect. Thus the electron withdrawal mechanism of the  $CF_3$  group does not seem to be due to  $\pi$  induction but due to field effect. It is interesting to note that a similar idea has been put forward by Streitwieser and Holtz to account for the differences in reactivity.

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# A MICRO SUBLIMATION STEP IN THE UV SPECTROPHOTOMETRIC DETERMINATION OF DIAZINON

THE microdetermination of diazinon<sup>1-3</sup> through 2-isopropyl-6-methyl-4-pyrimidinol is tedious; further the presence of animal and vegetable co-extractives complicate the analysis. In this communication a micro sublimation step is described for the purification of the pyrimidinol before UV determination.

2-isopropyl-6-methyl-4-pyrimidinol sublimes to white crystalline needles. The sublimation of the pyrimidinol is carried out by heating at  $115 \pm 1^\circ \text{C}$  for 10 minutes and is thus purified from co-extractives which in effect do not volatalise at this temperature.

The sublimation assembly (Fig. 1) consists of a metallic hot air-bath top covered with an asbestos sheet.

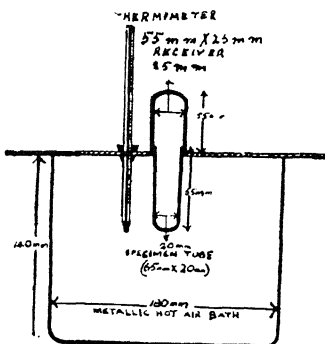


FIG. 1. Diagram showing the sublimation assembly.

An unsputted specimen tube fits the central hole of the asbestos cover, through another hole passes a thermometer. The receiver is another unsputted specimen tube of a slightly larger diameter. The bath may be heated either electrically or by flame at the rate of about  $10^\circ \text{C}$  per minute.

The contaminated pyrimidinol requiring purification is taken in the specimen tube, and the sublimation carried out at  $115 \pm 10^\circ \text{C}$  for 10 minutes and then the apparatus is allowed to cool to the ambient temperature. The sublimate is dissolved in 95% V/V ethanol and absorbance measures at 272 nm. The quantity of diazinon is then determined from a calibration curve prepared from purified pyrimidinol. One mg of pyrimidinol is equivalent to 1.657 mg of diazinon. The calibration curve obeys Beer's Law over the range of 0-80 ppm of pyrimidinol and the molar extinction coefficient is 4864.

For recovery studies known quantities of the pyrimidinol were spiked with about 500 fold excess

of tissue extractive and the recovery by sublimation determined. Recovery of the pyrimidinol by the proposed method was satisfactory and reproducible and was found to be 99.25% with a standard deviation of  $\pm 0.75\%$  upto  $100 \mu\text{g}$  level. Sublimation of quantities greater than  $100 \mu\text{g}$  results in substantial loss of the pyrimidinol and is therefore not recommended. It was found that a slight variation in the sublimation assembly did not significantly affect the recovery.

This purification step by micro sublimation should find an extensive application in the determination of diazinon in matrices such as animal tissue, vegetable matter, etc., giving copious co-extractives.

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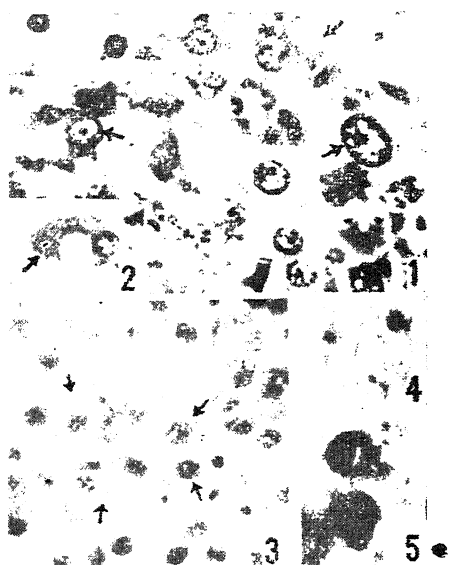
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## DISTRIBUTIONAL PATTERN OF THYROTROPHS IN THE PITUITARY OF HETEROPNEUSTES FOSSILIS (BLOCH.) BASED ON RADIOTHYROIDECTOMY

ALTHOUGH the pituitary of teleosts has been extensively studied<sup>6,1,7,8,4</sup> lack of agreement about the function of the various pituitary cell types is obvious. It is largely due to the fact that experimental allocation of function to the cells has been attempted systematically only in a few species<sup>1</sup>. In the teleosts the distributional pattern of the thyrotrophs greatly vary among different species. In some they are situated in the proximal pars distalis<sup>2</sup>, whereas in others they are in the rostral pars distalis<sup>3</sup> or even between the two zones<sup>5</sup>.

In the present investigation 10 *H. fossilis* were given  $250 \mu\text{Ci}$  of I-131 in two instalments at an interval of six months and sacrificed after the completion of a full year. The pituitary was fixed in Bouin's fluid containing 5 gm of Mercuric Chloride; sections were cut at  $5 \mu$  thick and stained in combination of PAS and lead haematoxylin (PbH), aldehyde fuchsin (AF) with Halmi's fastgreen-Chromotrope 2R-Orange G and Mallory's triple stain. Thyroid was fixed in Bouin's fluid, stained in PAS and Ehrlich haematoxylin and autoradiographs were prepared using NTB 3 Kodak emulsion.

In only one fish remnants of the thyroid was present in the form of 3 or 4 scattered micro-follicle which gave positive autoradiograph (Fig. 4). In the others as no viable thyroid tissue was present autoradiographs were negative. In the partial thyroidectomised fishes as the thyroid is in the form of microfollicle autoradiograph is very small when compared to the controls (Fig. 5). Both in partial and total radiothyroidectomised fishes the pituitary thyrotrophs exhibited extensive compensatory hypertrophy. They measure  $14\mu$  in diameter in comparison to control which measure only  $7\mu$  (Figs. 1 and 3). The activated nuclei is found with prominent nucleoli. Several of them have large vacuoles in their cytoplasm (Figs. 1, 2).



Figs. 1-5. Figs. 1, 2. Arrows show hypertrophied thyrotrophs in the radiothyroidectomised fish,  $\times 800$ . Fig. 3. Arrows show thyrotrophs in a normal fish,  $\times 800$ . Fig. 4. Autoradiograph of the thyroid in a partially thyroidectomised fish,  $\times 80$ . Fig. 5. Autoradiograph of three thyroid follicles in a normal fish  $\times 80$ .

These hypertrophied cells sometimes lack a distinct cell boundary and groups of them look like a syncytium. In the control specimens it is difficult to differentiate the thyrotrophs from the gonadotrophs as both were stainable with PAS, AF, aniline blue and fastgreen. In this species the thyrotrophs are localised in the middle of the proximal pars distalis flanked by gonadotrophs on either side. The acidophilic somatotrophs are distributed throughout the proximal pars distalis.

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#### EFFECT OF A COPPER INTRA-UTERINE CONTRACEPTIVE DEVICE ON SUB-CELLULAR DISTRIBUTION AND CONCENTRATION OF COPPER IN THE RAT UTERUS

COPPER containing intrauterine contraceptive device (Cu-IUD) has been reported to have better contraceptive efficacy and minimum side-effects than the polyethylene IUDs. The primary mode of action of copper has been attributed to its effect on endometrium and alteration of uterine fluid milieu both in animals and woman<sup>1</sup>. The catalase enzyme activity in the endometrium of rat is reported to be stimulated by Cu-IUD<sup>2</sup>. The presence of copper in cervical mucus inhibits sperm penetration<sup>3</sup>. There is non-uniform dissolution of copper ions from wire and even flakes of copper are shed from the wire<sup>4</sup> but the overall release rate of copper from device is  $10.3\text{ mg/year}^5$  and its concentration is significantly higher in the late secretory phase in woman<sup>6</sup>. It is pertinent to mention that copper content of liver, lungs, etc., remains unaltered after Cu-IUD signifying its local effect<sup>4</sup>. The subcellular localisation of copper in the uterus is still unknown.

Accordingly, the present investigation deals with the subcellular distribution of copper in rat uterus fitted with a copper IUD.

Colony bred adult female albino rats (150-200 gm) of the Institute with regular estrous cycle were used in this investigation. A piece of clean, soft, 100% copper wire (0.2 mm diameter) was fitted through the lumen of one uterine horn irrespective of the stage of the cycle by methods described previously<sup>2</sup>. The contralateral horn was sham operated by passing the suture needle through the uterine lumen and served as control. Surgery was carried out under aseptic precautions.

The animals were sacrificed 15 day after Cu-IUD insertion or sham operation during the estrus phase of the cycle. The copper-bearing and control uterine horns were carefully dissected out, pressed between filter papers to remove the luminal fluid and weighed to the nearest 0.2 mg on a Roller-Smith balance. Copper was estimated by the technique of Hubbard and Spettel<sup>7</sup> and polarographic estimations were done by the method described earlier<sup>8</sup>.

TABLE I

The uptake of copper ions ( $\mu\text{g/gm}$  wet-weight) by subcellular fraction of rat uterus

| Fractions    | Uterus          |                 |
|--------------|-----------------|-----------------|
|              | Control horn    | Cu-IUD horn     |
| Nuclear      | $1.57 \pm 0.14$ | $2.88 \pm 0.17$ |
| Mitochondria | $1.33 \pm 0.14$ | $1.30 \pm 0.10$ |
| Microsomes   | $0.65 \pm 0.11$ | $0.67 \pm 0.65$ |
| Supernatant  | $2.43 \pm 0.17$ | $4.00 \pm 0.12$ |
| Whole tissue | $6.43 \pm 0.16$ | $8.60 \pm 0.29$ |

Data is based on 6 estimations of each fraction.

It will be evident from the results presented in Table I that the concentration of copper was highest in both the horns in supernatant followed by nuclear, mitochondrial and microsomal fractions in a diminishing order. In the Cu-IUD bearing uterine horn, there was a significant increase in copper in nuclear and supernatant fractions ( $P < 0.01$ ) while its level remained virtually unaltered in mitochondrial and microsomal fractions. In the whole uterine tissue, also copper was significantly accumulated in Cu-IUD horn ( $P < 0.01$ ).

Copper is a versatile catalyst and participates in a number of enzyme reactions under normal physiological concentrations. A significant increase in the copper content of Cu-IUD bearing uterine horn appears to create a pharmacological condition and inhibits or blocks copper dependant enzymes, thereby making the endometrium hostile to reception of eggs. It is reported that continuous liberation of metal ions in the uterine lumen is necessary for their sustained contraception action<sup>9</sup>. Histochemically, copper has been demonstrated in the endometrium of women during the secretory phase<sup>10</sup>.

Thus the present study reveals that copper gets accumulated in the uterus and apparently alters its receptivity to eggs.

Studies are in progress to determine the release rate of copper in the uterine tissue and fluid.

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# OCCURRENCE OF STREPTOMYCIN RESISTANT AND DEPENDENT MUTATIONS IN *RHIZOBIUM LUPINI* UNDER THE INFLUENCE OF NITROUS ACID

RESISTANCE, dependence and sensitivity to streptomycin are well known in bacteria. Newcombe and Hawirko<sup>1</sup> isolated streptomycin dependent mutants of *Escherichia coli* in the presence of streptomycin. These mutants would not grow if streptomycin was not supplied to the medium. Balassa and Gabor<sup>2,3</sup> studied transformation using a streptomycin dependent strain of *Rhizobium lupini* which had been obtained as a single step spontaneous mutant. A streptomycin dependent strain was isolated by Mishra and Ravin<sup>4</sup> from a streptomycin resistant population of *Diplococcus pneumoniae*. In the present report isolation of a streptomycin dependent strain by the treatment of the streptomycin sensitive strain of *Rhizobium lupini* with nitrous acid is communicated.

A strain of *Rhizobium lupini* (H-13-3) obtained from the Hungarian Academy of Science,

budapest, Hungary, was used. Strain  $SM_1S_{10}$  was obtained as a spontaneous streptomycin resistant mutant from this strain. Strain  $NSD_1$  was a streptomycin dependent strain obtained as a result of nitrous acid treatment of sensitive strain H-13-3.

**Mutagenic treatment.**—Freshly growing cell suspension of *Rhizobium lupini* was diluted ten times in liquid complete medium as described by Balassa<sup>2</sup> and which also contained 1 ml of vitamin mixture, 2 ml of RNA hydrolysate and 1 ml of DNA hydrolysate per litre of medium and incubated overnight at 30° C. After washing with normal saline and resuspending in the same volume of normal saline the cells were treated with nitrous acid at a concentration of 0.017 M at pH 4.5 for 0 to 120 seconds. The reactions were terminated by adding 1 ml of the treated sample to 99 ml of complete liquid medium at pH 7.0 and incubated for 18 hours at 30° C. Platings were done on plain CM-agar and CM-agar containing streptomycin, immediately after treatment as well as after post treatment incubation. Colonies arising on antibiotic plates were further tested for their ability of resisting higher concentrations of the antibiotic.

From Fig. 1, it is evident that the percentage of survival decreases exponentially with the increase

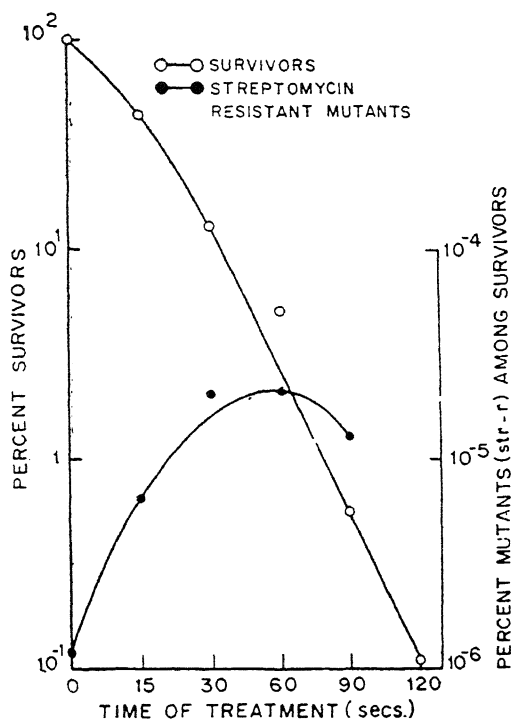


FIG. 1. Effect of nitrous acid on survival and mutation in *R. lupini* (strain H-13-3).

of time of treatment with nitrous acid whereas, the percentage of streptomycin resistant mutation did not show such type of relationship. In the case of streptomycin resistant mutation, the highest rate of mutation occurred at 60 seconds of nitrous acid treatment followed by post incubation after which the mutation rate decreased. It was concluded (Fig. 1) that streptomycin resistant mutations were highest when the killing was between 5 to 10%.

Only one streptomycin dependent strain designated as  $NSD_1$  was obtained at 60 seconds of treatment with nitrous acid followed by post-incubation for the expression of mutation, and all other colonies were streptomycin resistant.

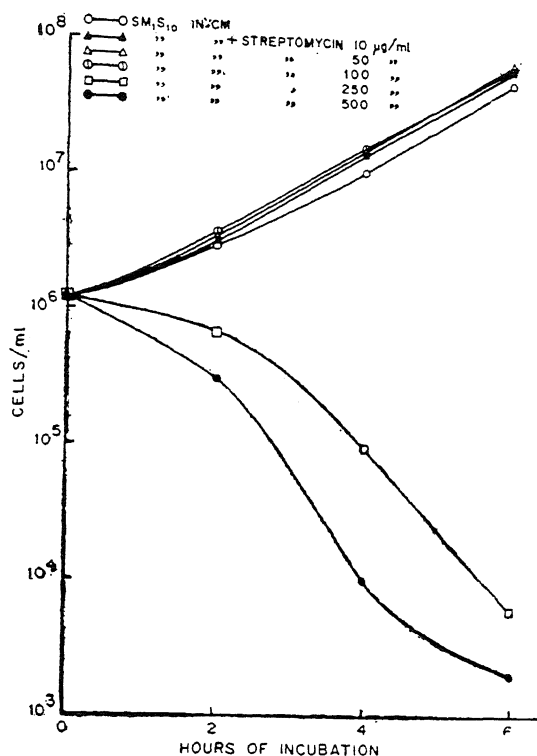


FIG. 2. Growth pattern of streptomycin resistant strain  $SM_1S_{10}$  at different concentrations of streptomycin.

**Growth pattern of streptomycin dependent strain as compared with streptomycin resistant and sensitive strains.**—For the study of the growth pattern of three strains streptomycin sensitive H-13-3, streptomycin resistant  $SM_1S_{10}$  and streptomycin dependent  $NSD_1$ , freshly growing cells in exponential phase were washed twice in normal saline and resuspended in liquid complete medium in a number of 50 ml Erlenmeyer flasks to which different concentrations of streptomycin were added.

One set for each strain was left without the addition of the antibiotic. Platings were done after 0, 2, 4 and 6 hours of incubation at 30°C and colonies were counted after 48 hours of incubation.

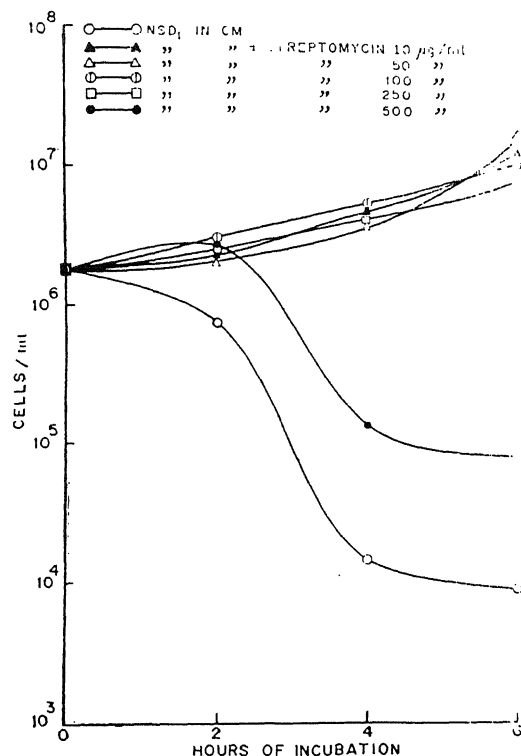


FIG. 3. Growth pattern of streptomycin dependent strain NSD<sub>1</sub> at different concentrations of streptomycin.

The complete inhibition of sensitive cell was more pronounced at higher concentration than at lower concentrations. Streptomycin sensitive strain H-13-3 showed killing effect in presence of only 10 µg/ml streptomycin and with higher concentrations none of the cells survived. Streptomycin dependent strain NSD<sub>1</sub> had difficulty in surviving in the absence of streptomycin (Fig. 3) proving thereby, that streptomycin was essential for its growth but strain H-13-3 and SM<sub>1</sub>S<sub>10</sub> had no such difficulty in the absence of streptomycin (Fig. 2). It has been established by Spotts and Stanier<sup>6</sup> that streptomycin causes some alterations in the structure of the ribosomes in such a manner that the dependent mutation hampers their ability to combine with certain types of messenger RNA. This may be repaired by the addition of streptomycin. The sensitive cells have great affinity for streptomycin which makes the ribosomes incapable of combining with messenger

RNA but the ribosomes of resistant cells have no such affinity, being unaffected by streptomycin. Both resistant and dependent strains showed increase in growth in the medium containing low concentrations of streptomycin but decrease was observed in the case of high concentrations thereby showing that most probably, in this case, the ribosomes were adapted only for the lower concentrations of the antibiotic and that the protein synthesis was hampered at higher concentrations of the antibiotic.

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#### A NEW RECORD FOR *CARIDINA BRACHYDACTYLA PENINSULARIS* KEMP, 1918 (DECAPODA, CARIDEA, ATYIDAE) FROM INDIA

THE present note reports the subspecies *Caridina brachydactyla peninsularis* Kemp for the first time from continental Asia beyond the Malayan peninsula. The subspecies is quite common in a perennial pond and neighbouring semi-permanent pools on the outskirts of Guntur (16°18' N and 80°29' E; MSL + 31.88 m) in Andhra Pradesh, India. The rain-fed pond also receives some water from river Krishna through an irrigation canal.

A random sample of forty-five specimens from material collected over the period March 1973 to July 1974 was utilised for biometric studies. The data were compared with those of Kemp<sup>1</sup>, as well as those of the syntypes in the British Museum (kindly sent by Dr. R. W. Ingle). There is general concurrence of the data.

The largest specimen examined is a berried female of TL 23.5 mm, CL 5.0 mm, RL 5.0 mm; the corresponding data for the largest male are 22.0, 4.0 and 4.5 mm. The rostrum (Fig. 1), which is of diagnostic importance, extends beyond the antennular peduncle and in some specimens, even a little beyond the antennal scale; its dorsal margin along its entire length is provided with a series of 31 to 46 teeth (usually 34-38), of which



2 to 4 lie on the carapace; the dorsal teeth are moveable, and a pair of setae are set between successive teeth. The ventral margin of the rostrum bears 6 to 15 teeth (usually 9-11); the distal part of the ventral border is unarmed: the ventral teeth are immovable, laterally compressed and without any setae between them.

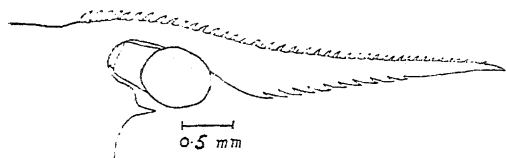


FIG. 1. *Caridina brachydactyla peninsularis* Kemp, 1918; rostrum (CL = 3.5 mm).

Body in general translucent; while minute orange, maroon and green chromatophores are scattered over the entire body, they are closer together along the posterior borders of the somites, on the telson and on uropods. Antennular peduncles and eye-stalks have larger green and deep maroon chromatophores.

The nominate subspecies *Caridina brachydactyla brachydactyla* De Man has been recorded from Indonesian Islands and Andaman Islands (Tiwari and Pillai<sup>2</sup>), from brackish as well as freshwaters. The subspecies *peninsularis* Kemp has been until now known only from the Malayan peninsula. The type material of Kemp<sup>1</sup> was from near Patani in Thailand and from a stream of clear water in the Botanic Gardens of Penang Island. Johnson<sup>3-5</sup> recorded it from Singapore, from some mainland streams as well as from tidal but non-saline waters.

It is of interest to note that near Guntur, the subspecies *peninsularis* has established itself in lentic bodies of freshwater not connected to an estuary. We have so far not come across this subspecies in the lower reaches of river Krishna. The shallow pond and pools which the subspecies inhabits are overgrown with *Typha*, *Vallisneria*, *Ottelia*, *Ceratophyllum* and other macrophytes; the shrimps occur among the vegetation.

We thank M/s. M. K. Durga Prasad and Y. Ranga Reddy, Research Fellows in this Department, for initially drawing our attention to the material. We are indebted to Dr. R. W. Ingle of British Museum (NH), London, for sending us relevant data of the syntypes. One of us (K. R.) thanks the authorities of CSIR, New Delhi, for the award of a Research Fellowship.

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#### A NEW SANGUINICOLID CERCARIA FROM *AMNICOLA TRAVANCORICA* IN ANDHRA PRADESH

WHILE studying the infestation of freshwater snails with intra-molluscan stages, a cercaria belonging to a rare and little known group, viz., 'Sanguinicolid cercariae' was recorded from Balacheruvu in Kakinada, Andhra Pradesh. The cercaria was found emerging from one out of 37 snails belonging to the species *Amnicola travancorica* (Benson). This is the first report of a freshwater sanguinicolid cercaria from this country.

The methods of study were the same as suggested by Cable<sup>1</sup>. Cercariae for measurement were killed in hot water. All measurements are in mm. Figures are camera lucida drawings of heat killed, well-relaxed cercariae under coverslip, with the details added free hand.

The cercaria (Figs. 1 and 2) is small and the body is covered with small spines. The spines are larger at the anterior end. Tail is ventrally attached to the body, such that the body is almost at a right angle to the tail. Furcal rami are short, pointed and devoid of fin-folds. Both the tailstem and rami are covered with very small spines. Six to eight long bristles borne on conical elevations are present along the sides of the tailstem. There is no dorsal crest on the body. The tip of the body does not form an anterior penetrating organ as in the case of spirorchiid and schistosomatid cercariae, the oral sucker being absent. However, the spines at the anterior end are bigger and closely arranged. A pair of hollow piercing spines, characteristic of blood flukes are present at the tip of the body. Ventral sucker is absent. Ventral mouth leads into a long, club-shaped oesophagus that extends to about one-half of the length of the body. Caeca are absent. There are a large number of unicellular glands in the body; whether these glands represent the penetration glands could not be ascertained, as the ducts were not clear.

Excretory system is mesostomate (Fig. 2). Excretory bladder is small. There are three pairs of flamecells in the body and none in tail. A single caudal excretory tubule has been observed in the present study, as also by several other workers, in

closely related aporocotyloid cercariae (Sewell<sup>2</sup>, Mc Coy<sup>3</sup>, Holliman<sup>4</sup>, etc.), while Ejsmont<sup>5</sup> represented two caudal excretory tubules in the sanguinicolid cercaria studied by him.

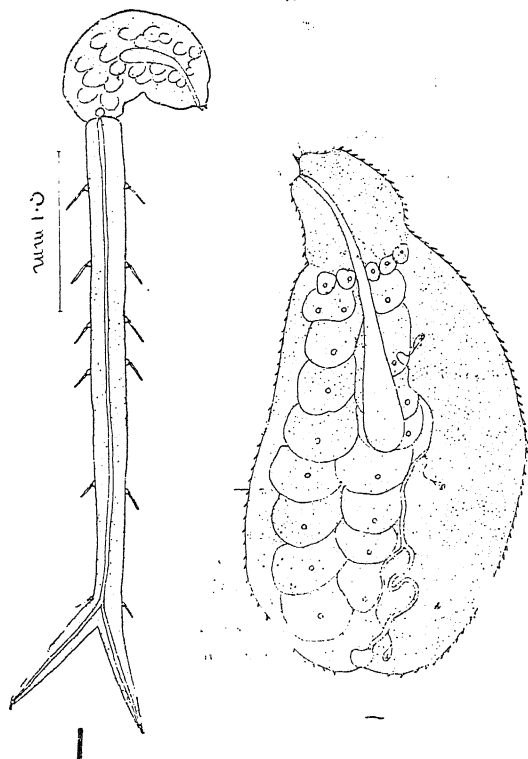


FIG. 1. *Cercaria indicae* LXVI. n.s.p., entire cercaria lateral view.

FIG. 2. Body of the cercaria, lateral view, showing excretory and digestive systems.

**Measurements.**—Body length 0.092–0.105 and width 0.05. Tailstem 0.29–0.312 × 0.02–0.026, rami 0.84 × 0.013.

Cercariae emerge in moderate numbers in a day. Swimming is sporadic. Prolonged periods of rest alternate with an occasional attempt to swim. Cercaria rests with the tailstem bent on itself, and the rami crossing the stem, forming a characteristic looped structure.

Intramolluscan stages could not be studied as the snail host perished while the cercaria was being studied.

*Sanguinicolid cercariae can be characterised, as follows:* "Apharyngeate, brevifurcate, non-ocellate; dorsal fin-fold on body and furcal fin-folds may or may not be present, tail symmetrical or asymmetrical, anterior organ reduced or lacking; ventral sucker present or absent. Develop in

marine lamellibranchs or freshwater snails. Cercariae penetrate directly into fishes and develop into adults in the vascular system or rarely in the coelom".

The present species differs from all the described sanguinicolid cercariae except *C. hartmanae* Martin, 1952, *C. amphictis* Oglesby, 1961, and cercaria of *Sanguinicola davisi* Wales, 1958 in the absence of a dorsal fin-fold on body and furcal fin-folds. The present species differs from *C. hartmanae* in the absence of an anterior organ and ventral sucker, from *C. amphictis* in having a brevifurcate tail and from cercaria of *S. davisi* in being smaller and in the nature of the gut (intestine in the cercaria of *S. davisi* is four lobed) and in the attachment of the tail to the body (tail being posteriorly attached to the body in the cercaria of *S. davisi*).

The present species is named *Cercariae indicae* LXVI in continuation of the numbers used for Indian cercariae by Sewell<sup>2</sup>.

I am greatly indebted to Prof. K. Hanumantha Rao for his valuable suggestions. Thanks are due to the authorities of C.S.I.R. and U.G.C. for financial support.

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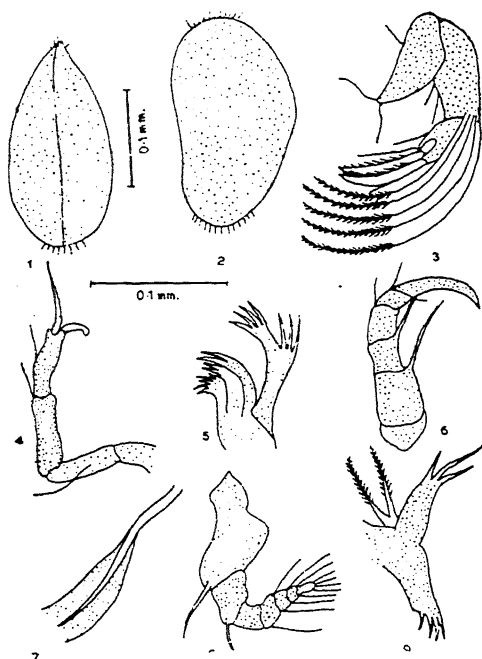
#### CYPRIDOPSIS OCHRACEA SARS, 1924 (CRUSTACEA : OSTRACODA), A NEW RECORD FROM INDIA

WHILE studying the zooplankton fauna of Bihar, one of the authors (SAKN) came across Ostracoda, collected from a freshwater pond at Bhagalpur, Bihar. The specimens were identified as *Cypridopsis ochracea* Sars. These specimens have been deposited in the museum of the Zoological Survey of India, Calcutta, and the museum of the Post-Graduate Department of Zoology, Bhagalpur University.

A review of the literature reveals that the species is a rare one, and the only known record is by G. O. Sars from South Africa. This species is not recorded from the Indian sub-continent. The present note is intended to place on record the

actual occurrence of the species of *Cypridopsis ochracea* Sars from the Indian sub-continent. Since no description of this species is available in the Indian fauna for ready consultation, a brief description of the animal is given below.

**Structure of the animal.**—The animal has a total length of 0.78 mm, the height 0.50 mm and the width 0.30 mm. Shell small moderately tumid in dorsal view, cuneiform anterior end gradually compressed and acutely pointed, posterior end obtusely rounded, maximum width not nearly attaining half the length and occur little behind the middle (Figs. 1 and 2). In profile, oblong and trigonal form with the greatest height somewhat in front of the middle and not fully attaining three-fifth of the length, dorsal edge angularly bent just behind the ocular region and declining rather steeply with the margin slightly sinuate, anterior end obliquely rounded and posterior somewhat produced below. Surface unsculptured with the fine scanty hairs near the extremities. The structure of the specimen resembles that of the *Cypridopsis ochracea* as reported by Sars (1924).



FIGS. 1-9. Fig. 1. Dorsal view female. Fig. 2. Side view female. Fig. 3. Second antenna. Fig. 4. Second abdominal leg. Fig. 5. Maxilla. Fig. 6. First abdominal leg. Fig. 7. Furcal rami. Fig. 8. First antenna. Fig. 9. Maxilliped.

**Remarks.**—*Cypridopsis ochracea* Sars, is easily recognisable from any other forms recorded, by

the shape of the shell and its unusual light yellow colour, which is retained in specimens for a longer time even after preservation in alcohol.

Our sincere thanks to Professor J. S. Datta Munshi, Head, Zoology Department, Bhagalpur University, for his constant help and encouragement, to the Director, Zoological Survey of India, for identification. One of the authors (SAKN) extends his thanks to the C.S.I.R., New Delhi, for the award of a Research Fellowship.

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### OCCURRENCE OF BIOVULAR FOLLICLES IN *HERPESITES JAVANICUS* AND *NYCTICEBUS COUCANG*

POLYOVULAR follicles and polynuclear oocytes have been reported in the ovaries of some mammals<sup>1-3</sup>. The present report embodies observations on the biovular follicles in *Herpestes javanicus* and *Nycticebus coucang*.

The ovaries of *Herpestes javanicus* and *Nycticebus coucang* were fixed in Bouin's fluid and 10% formalin. Paraffin sections were cut at 8 to 10  $\mu$  thick, and stained with Ehrlich's hematoxylin-eosin and periodic acid-Schiff procedure.

In *Herpestes javanicus* biovular follicles at the multilaminar follicular stage of development are found situated towards the periphery of the ovary. The two oocytes of the multilaminar biovular follicle (Fig. 1) are approximately equal in size, and are separated by the cells of membrana granulosa. Each oocyte of the biovular follicle has a single eccentric nucleus and is surrounded by a distinct PAS-positive zona pellucida. The cells of the membrana granulosa are round in shape, each having a darkly stained centrally placed nucleus. The ovary containing biovular follicles has a corpus luteum of early pregnancy. A few atretic follicles and a large number of primary oocytes are also present in the same ovary.

In *Nycticebus coucang* biovular follicles are situated towards the periphery of the ovary. The

two oocytes of the biovular follicle (Fig. 2) lie in direct contact with each other and are of equal size. Each oocyte of the biovular follicle has a centrally placed nucleus.



FIGS. 1-2. Fig. 1. Photomicrograph of a biovular follicle of *Herpestes javanicus*. Magnification on  $\times 220$ . Fig. 2. Photomicrograph of a biovular follicle of *Nycticebus coucang*. Magnification,  $\times 250$ .

In both these animals, biovular follicles are not seen beyond multilaminar follicular stage. It is likely that these follicles may undergo atresia. Whereas in *Herpestes javanicus* the two oocytes of the biovular follicle are separated by the cells of membrana granulosa, in *Nycticebus coucang* the two oocytes of the biovular follicle lie in direct contact with each other.

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# ON THE NUCLEAR POLYHEDROSIS OF *PLUSIA CHALCYTES* ESP. (LEPIDOPTERA: NOCTUIDAE)

NUCLEAR polyhedrosis has been reported in *Plusia gamma* L. by Lepine *et al.* (1953) and *P. chalcytes* Esp. by Laudeho and Amargier (1963). The present communication deals with the occurrence of nuclear polyhedrosis in larvae of *Plusia chalcytes* Esp. (= *P. eriosoma* Dbl.) feeding on groundnut (*Arachis hypogaea* L.) and *Flaveria australasica*. This appears to be the first record of nuclear polyhedrosis in *P. chalcytes* from India.

Infected caterpillars exhibited typical symptoms of nuclear polyhedrosis with rupturing of integument. On inoculation, the polyhedral suspension was found to be highly infective to third and fourth instar caterpillars. The incubation period of the virus in fourth instar caterpillars ranged from five to seven days. The inclusion bodies were somewhat triangular in shape (Fig. 1) and ranged from  $0.64$  to  $1.35 \mu$  in diameter with a mean of  $0.98 \mu \pm 0.01$ .



FIG. 1. Electron micrograph of polyhedra isolated from *P. chalcytes*. Line =  $0.674 \mu$ .

Paraffin sections of infected caterpillars revealed the presence of polyhedra in the nuclei of fat body, hypodermis, tracheal matrix, muscles, nerve sheath, hind gut, malpighian tubules and testicular epithelium. The nuclei of the midgut epithelial cells were found to be hypertrophied with some dark stained particles. This may indicate replication of the virus in the nuclei of the epithelial cells of the midgut as reported by Laudeho and Amargier (1963) in *P. chalcytes* but it requires confirmation electron microscopically.

The authors are grateful to Dr. Jean R. Adams, Research Entomologist, Insect Pathology Laboratory, Beltsville, Maryland, for making the electron micrograph and confirming the identity of the virus.

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### CROSS INFECTIVITY OF NUCLEAR POLYHEDROSIS OF *AMSACTA ALBISTRIGA* WALK. TO OTHER SPECIES OF LEPIDOPTERA

RESULTS of recent work on possible cross infection of polyhedrosis between closely related and unrelated Lepidoptera have been reviewed by Aizawa<sup>1</sup>, Huger<sup>2</sup> and Smith<sup>3</sup>, and it appears that insect viruses are far from being species specific as was once thought. Knowledge of the cross infectivity of insect viruses is of theoretical and practical importance, especially in the area of virus epizootiology and microbial control. This paper presents the results of inoculation tests with NPV of *Amsacta albistriga* Walk. (Arctiidae) on thirteen species of Lepidoptera.

Apparently virus free larvae of 1 to 5 days old were fed with NPV of *Amsacta albistriga* as inclusion bodies by contaminating the foliage of respective host plants with 10<sup>6</sup> polyhedra per ml. The insects tested were *Pericallia ricini* Fab. (Arctiidae), *Euproctis fraterna* Moore., *Notolophus posticus* Walk. and *Porthesia scintillans* Walk. (Lymantridae), *Earias vitella* Fab., *Spodoptera litura* Fab., *Heliothis armigera* Hub., *Orthago exivaneae* M. and *Cosmophila erosa* F. (Noctuidae), *Hyblaea parea* Cramer (Hyblaeidae), *Eupterote mellifera* Walk. (Eupterotidae), *Papilio demoleus* L. (Papilionidae) and *Sylepta derogata* Fab. (Pyralidae). The inclusion bodies were from semipurified stock of 5 to 7 months old. However, 100% infectivity was retained even after one year on *Amsacta albistriga*. The cause of death of test insects was diagnosed microscopically with squashed preparation. Cadavers having inclusion bodies in tissues were scored as virus-infected and those lacking inclusion bodies as negative.

It was evident from the results that nuclear polyhedrosis virus of *Amsacta albistriga* was not cross infective to the 13 species of Lepidoptera tested. Most of the test species died of bacterial

infection and of other unknown causes. The rest pupated normally without any pathological changes. No natural incidence of viruses were reported for the species tested except in *Spodoptera litura* (Nuclear polyhedrosis<sup>4</sup>), and *Pericallia ricini* (Nuclear polyhedrosis and granuloses<sup>5</sup>). Since these two species were not cross infective to NPV of *Amsacta albistriga* the possibility of induction of latent virus infection was ruled out. The negative results agree with older view of Steinhaus<sup>6</sup> that a high degree of host specificity is a characteristic of insect viruses.

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### EFFECT OF CROP AGE ON FIBRE QUALITY (TENACITY) IN JUTE (*CORCHORUS* SP.)

ALTHOUGH the jute crop is generally harvested soon after formation of pods, earlier harvesting is often found necessary, particularly for fitting it in a suitable crop rotation. While there is evidence of reduction in fibre yield when early harvesting is resorted to, not much information is available with regard to its effects on fibre quality. The present study was undertaken at the Block Seed Farm, Singur, Hooghly, in 1972, with a number of *C. olitorius* types and was repeated at the Seed Multiplication Farm, Chinsurah, in the same district, in 1973, to ascertain how far the tenacity of fibre, a very important quality character, is affected by advancing the date of harvest. The layout and different cultural operations were similar in both the years, but the retting conditions were a little different, stagnant water being used in the former and slow moving water in the latter. In the second year, a similar separate experiment was undertaken with types of *C. capsularis*.

In 1972, 23 types of *C. olitorius* were sown in two replications, each unit plot consisting of a single row of 1 metre length having 15 plants. Three plants were harvested at random from each

row at crop ages of 90, 100, 110 and 120 days. The tenacity of fibre of each type was determined with the help of a 'bundle tester', as is done at the Jute Technological Research Laboratories, Calcutta. In 1973, the study was repeated with 21 of these types along with 5 others, and one extra harvest at 130 days' crop age was investigated. In addition, 23 *capsularis* types were tested separately.

Typical values of fibre tenacity are given in Tables I and II to indicate the nature of the results.

TABLE I

Average fibre tenacity of *olitorius* types (gm./tex)

|                   |      | 90<br>days | 100<br>days | 110<br>days | 120<br>days | 130<br>days |
|-------------------|------|------------|-------------|-------------|-------------|-------------|
| 1. JRO-632        | 1972 | 34.20      | 27.10       | 21.50       | 16.60       | ..          |
|                   | 1973 | 34.31      | 32.06       | 28.93       | 26.81       | 15.12       |
| 2. JRO-620        | 1972 | 34.10      | 22.70       | 20.40       | 14.60       | ..          |
|                   | 1973 | 31.62      | 24.68       | 22.81       | 19.52       | 16.06       |
| 3. C.G.           | 1972 | 30.00      | 21.70       | 16.40       | 12.60       | ..          |
|                   | 1973 | 30.93      | 23.50       | 22.93       | 20.18       | 13.06       |
| 4. JRO-7835       | 1972 | 28.10      | 24.30       | 18.10       | 15.40       | ..          |
|                   | 1973 | 28.87      | 28.75       | 27.18       | 19.62       | 15.37       |
| 5. JRO-878        | 1972 | 24.90      | 23.90       | 22.10       | 14.30       | ..          |
|                   | 1973 | 29.87      | 29.81       | 29.37       | 23.18       | 15.18       |
| 6. Sudan<br>Green | 1972 | 27.90      | 26.00       | 19.40       | 15.20       | ..          |
|                   | 1973 | 29.75      | 23.87       | 23.00       | 22.75       | 14.00       |
| 7. R-26           | 1972 | 29.30      | 27.50       | 22.30       | 17.30       | ..          |
|                   | 1973 | 27.68      | 27.56       | 25.00       | 16.00       | 14.87       |
| 8. Tanganika-I    | 1972 | ..         | ..          | ..          | ..          | ..          |
|                   | 1973 | 30.87      | 30.25       | 30.87       | 28.50       | 23.31       |

## Tenacity Grades

1. Very good — above 31.0
2. Good — between 27.1 and 31.0
3. Fairly good — between 23.1 and 27.0
4. Average — between 19.0 and 23.0
5. Bad — below 19.0

TABLE II

Average fibre tenacity of *capsularis* types (gm./tex)

|                | 90<br>days | 100<br>days | 110<br>days | 120<br>days |
|----------------|------------|-------------|-------------|-------------|
| 1. JRC-321     | 19.5       | 26.1        | 24.4        | 23.8        |
| 2. D-154       | 19.5       | 25.1        | 22.8        | 15.0        |
| 3. JRC-212     | 18.6       | 25.4        | 19.8        | 11.2        |
| 4. JRC-201     | 20.9       | 25.7        | 20.5        | 16.1        |
| 5. JRC-1108    | 21.3       | 26.1        | 19.1        | 16.7        |
| 6. JRC-7447    | 20.5       | 23.6        | 18.2        | 15.1        |
| 7. Narrow leaf | 19.3       | 24.6        | 23.3        | 19.9        |
| 8. Zaoping-2   | 20.1       | 25.6        | 13.5        | 12.7        |

## Tenacity Grades

1. Very good — above 29.0
2. Good — between 25.1 and 29.0
3. Fairly good — between 21.1 and 25.0
4. Average — between 17.0 and 21.0
5. Bad — below 17.0

It is seen in Table I that, despite somewhat erratic performance of certain types, the tenacity of fibre, on the whole, decreased with delay in harvesting. While the types barring only a few were good or

very good in tenacity at the crop age of 90 days, there was a fall in each case as the date of harvest was deferred, and, at the last harvest (at 120 days and 130 days in 1972 and 1973 respectively), almost all the types, irrespective of their earlier performance, were found to be bad (tenacity being below 19). Even the types, which recorded very high tenacity values (above 31) at 90 days, registered in many cases fairly big fall with a delay in harvesting. The performance of *capsularis* types (Table II) was more or less similar to that of *olitorius* in so far as the decrease in tenacity of fibre with delay in harvesting was concerned. However, unlike the latter, the former showed an increase in tenacity at 100 days' age, after which the decrease actually started. This indicated that, while in *olitorius*, the first date, i.e., 90 days' crop age was found to be the best of all the dates considered, in *capsularis*, the second date, i.e., 100 days' crop age, was the best. This decrease in tenacity of fibre in both the species as the harvesting was delayed was probably due to the adverse effects of increasing age. In the case of *olitorius*, the correct period appears to be 90 days or even less and in the other species, 100 days was found to be the best.

Of the *olitorius* types studied, Tanganyika-I, which was studied in the second year only, recorded almost the same value till the third harvest, there being a slight fall in the fourth. Even at this stage, there was a little deterioration in tenacity. It was only at the age of 130 days that an appreciable decrease was observed and the tenacity was found to be fairly good. JRO 878, which was studied in both the years, showed considerable differences between the tenacity values of the two years at a particular crop age. However, the data, particularly of the second year, indicated that the variety was quite stable till the age of 110 days. In the case of *capsularis*, only one of the types, JRC 321, showed some stability till the last harvest.

The tenacity values, recorded in the second year at the crop age of 130 days, appeared on the whole, more or less similar to that of the first year at 120 days' age (Table I). The reasons for this difference can perhaps be traced to the difference in retting conditions. It may be that the quality of fibre of the later charges in the first year was adversely affected because the same water was used over and over again.

A detailed study is proposed to be taken up shortly to ascertain the exact reasons for deterioration in fibre tenacity of jute with an increase in crop age.

Thanks are due to the Director, Jute Technological Research Laboratories, Calcutta, and the Director

of Agriculture, West Bengal, for providing the necessary facilities.

All India Coordinated  
Research Project on Jute and  
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### A REPORT ON TRANSITION IN THE STOMATAL SIZE WITH AGING OF LEAVES IN *CLEOME VISCOSA* LINN.

POLYMORPHISM in the stomata has been recorded in a few plants<sup>1-4</sup>. During survey of stomata in arid zone plants, it was discovered that there is a distinct variation in stomatal size of *Cleome viscosa*. This is a very common plant growing wild in the rainy season. Plants were demarcated from different habitats under different conditions in the Jodhpur University campus. Three types of leaves were graded according to their maturity or aging (young, adult and old). Epidermal peelings were made from the abaxial surface of the leaf and stomatal measurements were made with the help of precalibrated microscope. The measurements are tabulated in Table I.

number of epidermal cells with the maturity of the leaves. Epidermal cells form a beak like structure on both the edges of stomata. Stomata with a single functioning guard cells were also

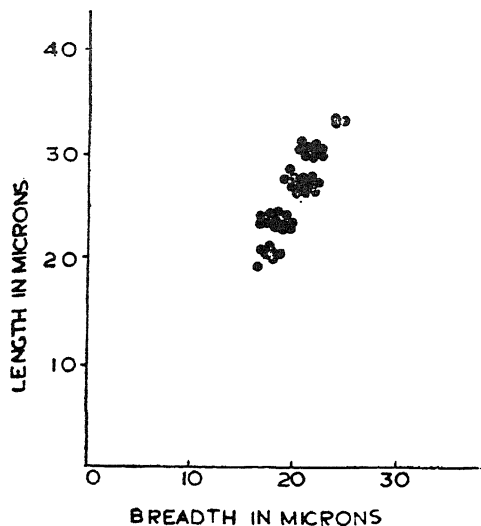


FIG. 1

TABLE I

Size of stomata, the epidermal content and the stomatal index of leaves of *C. viscosa*

| Age of leaf | No. stomata<br>per<br>sq.mm | Size of stomata in microns |          |          |          |          |          |          |          | No. epi. cells<br>per<br>Sq.mm | Stomatal<br>index |
|-------------|-----------------------------|----------------------------|----------|----------|----------|----------|----------|----------|----------|--------------------------------|-------------------|
|             |                             | 1                          |          | 2        |          | 3        |          | 4        |          |                                |                   |
|             |                             | <i>l</i>                   | <i>b</i> | <i>l</i> | <i>b</i> | <i>l</i> | <i>b</i> | <i>l</i> | <i>b</i> |                                |                   |
| Young       | 833±61                      | 27                         | 21       | 24       | 18       | 21       | 18       | 18       | 15       | 2320±243                       | 26.4              |
| Adult       | 593±54                      | 30                         | 21       | 27       | 21       | 24       | 18       | 21       | 18       | 2070±193                       | 22.3              |
| Old         | 340±46                      | 33                         | 24       | 30       | 21       | 27       | 21       | 24       | 18       | 1293±154                       | 20.8              |

*l*=Length of stomata; and *b*=Breadth of stomata.

It is evident from Table I that the number of stomata and epidermal cells per unit area decrease with the maturity or aging of leaves. Old leaves have fewer stomata and epidermal cells as compared to young ones. It is a well known fact that stomata continue to develop through a considerable part of the epidermal extension of the leaf by cell enlargement<sup>5</sup>, and new stomata would hardly arise when a leaf has attained full maturity. As per stomatal measurements, the stomata appear to fall in more than one group. It may appear that hardly new stomata arise in a mature leaf. Stomatal measurements at random show four distinct groups. The smallest stomata were seen only in the young leaves and the largest in the old ones only. Stomata of four sizes were seen common in all ages of leaves. The number of stomata of size  $27 \times 21 \mu$  and  $24 \times 18 \mu$  were common to all ages of leaves (Fig. 1). This indicates that there is an increase in the size of stomata and decrease in the

observed. Thus from the present study a correlation can be made between: (1) the number of stomata and number of epidermal cells; (2) number of stomata and maturity of leaf; (3) size of the stomata and maturity of leaf in this species.

The authors are thankful to Prof. H. C. Arya, Head, Department of Botany, for providing necessary facilities.

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Jodhpur (India), September 8, 1974.

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ON THE IDENTITY OF *HABENARIA*  
*GRANDIFLORIFORMIS* BLATT. McC.

*Habenaria grandifloriformis* Blatt. McC. and *H. grandiflora* Lindl. are considered to be duplicates taxonomically on the basis of similarities in the floral structure (Santapau and Kapadia, 1966). It was the hope of the author to evaluate the position of these species on their growth habits, morphology and anatomy of the vegetative organs.

The plants possess a very short stem that emerges out from a globose-ellipsoid special tuberous mother root, bearing usually a single leaf lying flat on the ground (Fig. 1). The stem bears a few short but stout roots and one among them has a swollen tip and forms the special tuberous root. In all the special tuberous roots studied, a single bud or two buds are initiated endogenously at the basal part of the tuber enclosed within a cavity. At a later stage these bud initials enlarge and get exposed as small protuberances.

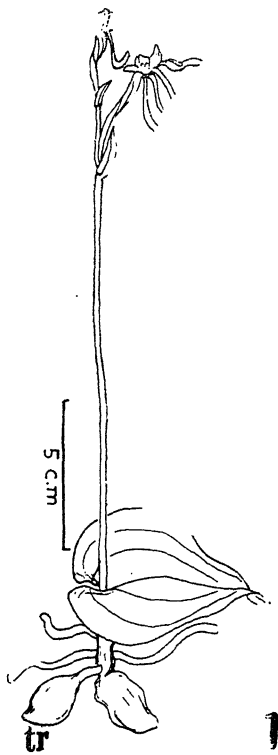


Fig. 1. *Habenaria grandifloriformis* Blatt. McC., entire plant; tm, tuberous mother root; tr, tuberous root.

Towards the end of the season the stem along with the wrinkled spongy special tuberous root remains underground, alive till the next monsoon when it develops a new plant. Then this special

tuberous root turns out to be the special tuberous mother root of that plant. This growth habit is repeated in the following seasons.

The specimens collected for the present investigation befit the taxonomical descriptions of both *H. grandifloriformis* and *H. grandiflora*, and revealed identical features of the corresponding vegetative organs. The morphology and anatomy of the vegetative organs, viz., root, special tuberous roots, stem and leaf, as brought out by this study, invariably show striking similarities between the two plants and a distinction of the two species becomes impossible.

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NEW RECORD OF LONG GRAIN IN RICE

VARIATION in grain length in rice has been reported by number of workers. The longest (14.23 mm) grain was obtained in Ac. 27 an introduction from China<sup>1</sup>. The variety SML 128/4 from Surinam had been reported to have a grain length of 14.40 mm<sup>2</sup>.

During a study carried out at the Central Rice Research Institute, Cuttack, on grain size, two long grained varieties Ac. 27 and Roti (grain length—13.15) from Madhya Pradesh were crossed in Kharif—1972.

The average grain length of Ac. 27 is 14.19 mm (range—13.98 to 14.50 mm). The grain length of Roti ranged from 12.68 to 13.58 mm with a mean of 13.15 mm. In the  $F_1$  plants the grains were intermediate in length (13.25 mm). In the  $F_2$  generation grain length ranged from 9.57 to 16.17 mm. Out of 530 plants studied 8 were in the transgressive class for longer grain. These 8 plants were carried over as plant progenies and in the  $F_4$  generation, one culture possessing average grain length 16.09 mm, was isolated. This was further studied in  $F_5$  and found to breed true. The grain development is normal. This is now being maintained. The detailed information about this culture as well as the parents is furnished in Table I.

The variation in grain type in  $F_2$  is shown in Fig. 1 along with that of  $F_1$  and parents.

Grain length exceeding 14.4 mm had not been reported earlier and the present observation is first of its kind. The test weight (1000 grain) was, however, less than Ac. 27 and this may probably be due to reduced breadth and thickness in addition



TABLE I  
Information on parents and extract

| Variety                | Maturity period (days) | Plant height (cm) | Tiller/plant | Panicle length (cm) | Grain/panicle | Grain dimension |              |                | Wt. of 1000 grains (gm) |
|------------------------|------------------------|-------------------|--------------|---------------------|---------------|-----------------|--------------|----------------|-------------------------|
|                        |                        |                   |              |                     |               | Length (mm)     | Breadth (mm) | Thickness (mm) |                         |
| Roti                   | 124                    | 118               | 9            | 29.5                | 120           | 13.15           | 3.35         | 2.39           | 42.0                    |
| Ac.27                  | 120                    | 108               | 7            | 24.0                | 75            | 14.19           | 3.46         | 2.39           | 48.5                    |
| Extract (Roti × Ac.27) | 120                    | 116               | 7            | 26.0                | 85            | 16.09           | 3.33         | 2.33           | 46.0                    |

CD at 5% for grain length 0.64 mm.

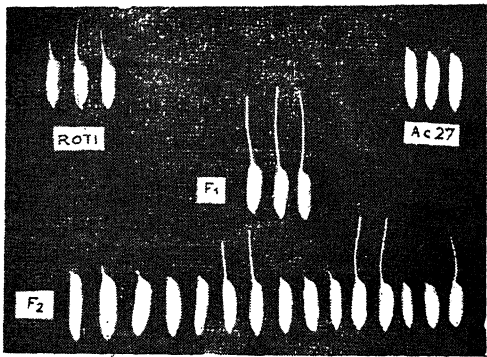


FIG. 1. Variation in grain type in F<sub>2</sub> along with that of F<sub>1</sub> and parents.

to other characters which normally influence the test weight.

Thanks are due to Dr. R. Seetharaman, Head, Division of Genetics, for encouragement.

Central Rice Research Institute, K. PRASAD.  
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September, 2, 1974.

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KARYOTYPE STUDY IN  
DIPCADI CONCANENSE DALZ.

THE genus *Dipcadi* is chiefly an African genus and in India eight species are reported to occur. All the Indian species flower in June and July and disperse seeds by August.

The cytology of the Indian species is not known in sufficient detail except for the report of the karyotypes in three species by Mahabale and Chennaveeraiah<sup>2</sup>. In the present paper the chromo-

somes number and karyotype of *D. concanense* is given and this is the first record. The material for the present study was collected by Prof. M. S. Chennaveeraiah from Malwan (Maharashtra). This species is apparently endemic and is distinguished from the rest by its large white flowers. The root tips were excised, pretreated with 0.002 molar 8-hydroxyquinoline for 4 hours at 10–15° C and squashed in aceto-orcein. For the description of karyotype the method followed by Levan *et al.*<sup>1</sup> has been adopted. For determining the type of chromosome the centromeric index  $r = (l/s)$ . Where  $r$  as the arm ratio,  $l$  as the length of the long arm and  $s$  as the length of the short arm is taken into account.

Twelve chromosomes were counted from the root tip cells. Somatic metaphase chromosomes and the idiogram are shown in Figs. 1–2. There are



FIGS. 1–2. Fig. 1. Photomicrograph of somatic metaphase (2n = 12) in *Dipcadi concanense*, × 1800. Fig. 2. Idiogram of the above.

3 pairs of long and 3 pairs of short chromosomes. Two pairs of long chromosomes have satellites on their short arms. Excepting one pair in which the centromere is terminal, in all the other pairs of chromosomes the centromere is submedian. The details of the karyotype are given in Table I.

TABLE I

Karyotype analysis in *Dipcadi concanense* Dalz.

| Chromosomes | Length in microns |               | Arm ratio | Centromere |
|-------------|-------------------|---------------|-----------|------------|
|             | Long arm          | Short arm     |           |            |
| I           | 5.50              | 2.20<br>+0.55 | 2.50      | sm SAT     |
| II          | 4.68              | 2.48<br>+0.99 | 1.82      | sm SAT     |
| III         | 6.60              | 0.83          | 7.95      | t          |
| IV          | 2.20              | 0.83          | 2.65      | sm         |
| V           | 1.79              | 0.83          | 2.15      | sm         |
| VI          | 1.51              | 0.83          | 1.81      | sm         |

It is significant to note that Mahabale and Chennaveeraiah<sup>2</sup> have also observed two pairs of satellited chromosomes in all the three species they have studied, and this is indicative of evolutionary relationships in the genus. Further, in this species although the chromosome number is the same as that of *D. saxorum*, the karyomorphology is entirely different from it. Both the species have very restricted occurrence.

Department of Botany, B. N. KANMANI.  
Karnatak University,  
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#### A NEW STOMATAL TYPE

THIS note reports a type of stoma which doesn't find a place, either in Metcalfe's (1950)<sup>2</sup> classification, or in the more recent one of Fryns Classaens and Van Cotthem (1973)<sup>1</sup> and suggests a name for it, for inclusion in the latter classification.

The author, in his studies on epiphytic orchids<sup>3,4</sup>, encountered stomata with three subsidiary cells, all of which are more or less of equal dimensions. He has observed them, also in *Aerides crispum* Lindl. *Porpax jerdoniana* (Wt.) Rolfe. The nearest type to this type of stoma, in Metcalfe's classification, is the 'anisocytic', which becomes 'anisomesogenous' or 'anisomesoperigenous' in Fryns Classaens and Van Cotthem's classification, in all three of which there are three subsidiary cells, out of which one is distinctly smaller than the other two. The author suggests the term 'isomesoperigenous', as a new type, for a three subsidiary-celled stoma, where all the three cells are of equal dimensions, as exemplified by the aforementioned plants, to be included

in the latter classification, under the mesoperigenous types.

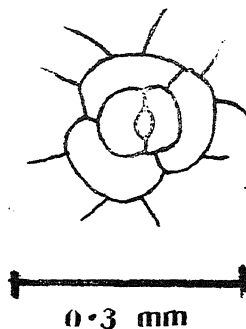


FIG. 1. An isomesoperigenous stoma from the leaf epidermis of *Aerides crispum* Lindl.

However, the recent classification too does not solve the basic problem, viz., the identity of the subsidiary cell, in epidermes where ordinary epidermal cells and those in the vicinity of the stomata, look alike morphologically, at maturity. It is possible to presume that they are truly subsidiary (in the ontogenetic sense), if there were but only one ontogenetic method of a particular type of stoma being developed, which need not necessarily be the case always.

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#### FUNGISTATIC PROPERTIES OF SOME SEEDLING EXTRACTS

THE tender seedling stage of a higher plant appears most vulnerable to infection by pathogens. But a large number of plants are able to escape infection, thereby indicating the presence of some fungistatic substance(s) in them. It was this aspect which prompted us to undertake the screening of seedlings for their fungistatic activity.

Seeds of various species (Table I) were allowed to germinate on moist filter paper in sterilized petriplates for two to three weeks and the seedlings were tested for fungistatic properties. Entire seedlings were crushed thoroughly to extract the juice, which was tested immediately against *Cephalosporium sacchari* Butl. and *Fusarium nivale* (Fries)

Cesati, by slide germination technique<sup>1</sup>. Percentage of spore inhibition was noted after 24 hours and the fungistatic activity was measured in terms of this percentage.

TABLE I  
Inhibition of spore germination by seedling extracts

| S. No. | Genus and species  | % inhibition of spore germination |                        |
|--------|--|-----------------------------------|------------------------|
|        |  | <i>Cephalosporium sacchari</i>    | <i>Fusarium nivale</i> |
| 1      | <i>Allium cepa</i> L.  | 1.00                              | 0.00                   |
| 2      | <i>Abelmoschus esculentus</i> L.                             | 30.96                             | 90.7                   |
| 3      | <i>Brassica campestris</i> L.<br>var. <i>dychotoma</i> Watt. | 100.00                            | 98.91                  |
| 4      | <i>B. campestris</i> L.<br>var. <i>sarson</i> Prain.         | 100.00                            | 100.00                 |
| 5      | <i>B. juncea</i> L.  | 0.00                              | 0.00                   |
| 6      | <i>B. oleracea</i> L. var <i>capitata</i> L.                 | 100.00                            | 100.00                 |
| 7      | <i>B. pekinensis</i> Lour.                                   | 0.00                              | 0.00                   |
| 8      | <i>B. rapa</i> L.  | 100.00                            | 36.50                  |
| 9      | <i>Cajanus cajan</i> L.                                      | 1.00                              | 0.00                   |
| 10     | <i>Cassia auriculata</i> L.                                  | 4.40                              | 100.00                 |
| 11     | <i>C. occidentalis</i> L.                                    | 3.80                              | 32.70                  |
| 12     | <i>Calotropis procera</i> Ait.                               | 0.00                              | 1.50                   |
| 13     | <i>Carica papaya</i> L.                                      | 45.00                             | 52.00                  |
| 14     | <i>Cicer arietinum</i> L.                                    | 10.00                             | 0.00                   |
| 15     | <i>Cucumis sativus</i> L.                                    | 2.00                              | 1.50                   |
| 16     | <i>Cucurbita moschata</i> Duch.                              | 36.00                             | 16.30                  |
| 17     | <i>Cyamopsis tetragonoloba</i> L.                            | 17.82                             | 15.12                  |
| 18     | <i>Dolichos lablab</i> L.                                    | 0.00                              | 99.80                  |
| 19     | <i>Ipomea fistulosa</i> L.                                   | 11.50                             | 6.70                   |
| 20     | <i>Lycopersicon esculentum</i><br>Mill                       | 0.00                              | 100.00                 |
| 21     | <i>Luffa acutangula</i> Roxb.                                | 12.00                             | 0.00                   |
| 22     | <i>Luffa cylindrica</i> Roem.                                | 57.20                             | 16.91                  |
| 23     | <i>L. echinata</i> Roxb.                                     | 14.00                             | 11.00                  |
| 24     | <i>Lagenaria siceraria</i> Staudl.                           | 36.80                             | 0.00                   |
| 25     | <i>Momordica charantia</i> L.                                | 7.50                              | 29.55                  |
| 26     | <i>Pisum sativum</i> L.                                      | 0.00                              | 14.00                  |
| 27     | <i>Raphanus sativus</i> L.                                   | 100.00                            | 100.00                 |
| 28     | <i>Tridax procumbens</i> L.                                  | 0.00                              | 100.00                 |
| 29     | <i>Vigna sinensis</i> L.                                     | 39.21                             | 30.00                  |
| 30     | Controi  | 99.98                             | 100.00                 |

Out of 29 taxa screened, the extract of four showed strong fungistatic activity against both test fungi (Table I). The extracts of six other species were partially active, inhibiting spore germination of either of the two test fungi. Surprisingly the extracts of *Brassica juncea* L. and *Brassica pekinensis* Lour. (5 and 7 in Table) stimulated the germination of fungal spores.

Table also shows that fungistatic property is neither a family character nor a generic one. It varies from family to family, from genus to genus

from species to species. A plant species which partially inhibited one fungus was found to be completely inhibitory to other one.

Further study is needed to isolate the fungistatic substance(s) from these seedling extracts. The authors are thankful to Prof. K. S. Bhargava, for providing laboratory facilities and encouragement to Dr. R. R. Upadhyay, for various suggestions and to the S.C.S.I.R., (U.P.), for financial assistance.

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### THE GENUS *PSEUDOBOTRYTIS* KRZEMIENIEWSKA AND BADURA

THE genus *Pseudobotrytis* was created by Krzemieniewska and Badura (1954)<sup>1</sup> with the type species *P. fusca*. Timonin (1940)<sup>4</sup> and Morris (1955)<sup>2</sup> described the same fungus as *Spicularia terrestris* Timonin and *Umbellula terrestris* (Timonin) Morris respectively. Subramanian (1956)<sup>3</sup> found that *P. fusca* K & B. and *U. terrestris* (Timonin) Morris were identical and congeneric and, therefore, he proposed a new combination *Pseudobotrytis terrestris* (Timonin) Subram. In 1961 Tironin<sup>5</sup> isolated from soil in Honduras a new species of this genus, *P. bisbyi* Timonin.

Recently the authors collected dead twigs bearing black fungal growth from Mt. Abu. The present fungus closely resembles *P. bisbyi* Timonin except that the conidiophores are short. *P. bisbyi* has not so far been recorded from other parts of the world and hence this is the first record of this fungus from India.

#### *Pseudobotrytis bisbyi* Timonin

Fungus forming effuse, blackish growth on the dead twigs. Repent vegetative hyphae brown, branched, septate, upto  $5.5\mu$  broad, producing 1-2 rhizoid like hyphae at the point of origin of conidiophores. Conidiophores arising laterally from cells of the repent hyphae, length upto  $120\mu$ , unbranched, simple, erect, straight, septate, distance between septa upto  $27\mu$ , dark-brown with the apical cell  $20-24.5 \times 4.0\mu$  being hyaline, slightly tapering towards the tip, breadth at the base  $5.4-6.7\mu$ , breadth at the middle  $4.7-5.4\mu$ ; tip globose,  $6.7 \times 5.4\mu$ , denticulate and bears a verticil of about 12 simple, hyaline, sterigma-like branches of nearly equal length,  $21.6-27.5 \times 2.5-4.0\mu$ , thick at the base, and gradually tapering towards the apex, finally each of which in turn terminates in

a nearly globose to elliptical tip. diameter of the tip  $6-6.7 \mu$ , bearing many conidia produced singly on minute, short pegs. Conidia ovate to oblong, 1-celled, pale-brown to fuliginous, smooth-walled, with a minute basal scar indicating the point of attachment,  $5-6.7 \times 3-4.7 \mu$  (Fig. 1).

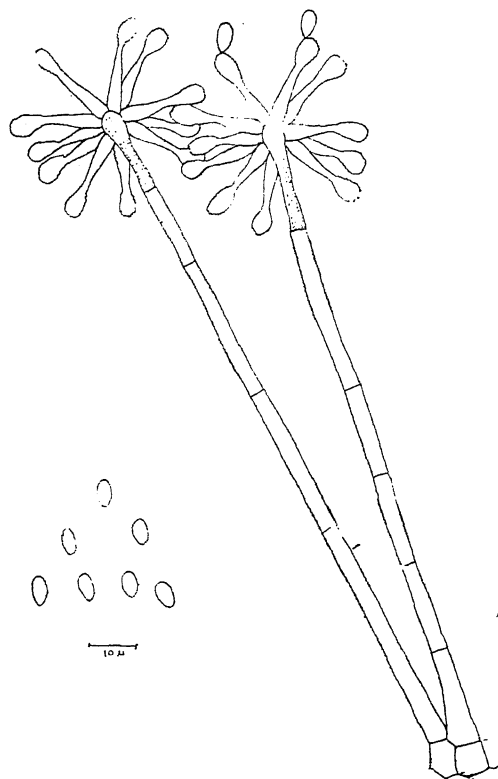


FIG. 1. Showing Conidiophores and Conidia.

Specimen deposited with Botany Department,  
University of Jodhpur, Jodhpur, J.U.M.L., 326.

Mycology and Plant Pathology      K. S. PANWAR.  
Laboratory,                                      J. S. CHOUHAN.  
Department of Botany,  
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# AN INTERESTING ASCOMYCETE FROM COORG (KARNATAKA)

DURING a tour in the Coorg Forests an interesting ascomycetous fungus was collected growing on the twigs of *Lobelia trigona* Roxb. The infected areas were in the nature of stroma forming a thin, black, carbonaceous outer layer on the host. Critical examination of the material further revealed its identity as a species of *Pleuroceras* Riess. (Diaporthaceae)<sup>1</sup>, hitherto an unrecorded genus in India. For its identity, the fungus was studied in detail and found to be distinct, differing significantly from *Pleuroceras cryptoderis* (Lev.) v. Hohn.<sup>2</sup>, the type species, as well as *P. populi* Thompson<sup>2</sup> in respect of gross-morphological characters and dimensions of fruiting structures, besides being collected on a new host. Hence, the same has been described here as a new species, viz.,

*Pleuroceras lobelii* sp. nov. (Fig. 1)

Stromata nigra, levia, coriaceo-carbonaceis tenui; perithecia rostellata, tenuiter-tunicata, membranaceo, defixus oblique in plantis textus, ostiolata, usque ad 1-2 per stroma, magnit:  $340-510 \times 255-340 \mu$ ; collo brevia vel piriformibus,  $136-170 \times 80 \mu$ , diagonalia et lateralia oriunda; periphyses numerosae, filiformae hyalinae; paraphysibus nullae; asci octospori, unitunicati, apophysatae, cylindracei vel clavati, emergentes in fasciculo, magnit:  $95-114 \times 9.5-11.4 \mu$ ; ascospores bicellulares, hyalinae, tenuiter-tunicati, forma fusus, constrictae ad septa,  $22.8-30.4 \times 2-3.8 \mu$ .

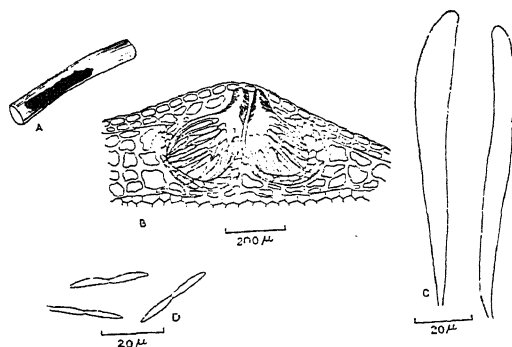


FIG. 1. *Pleuroceras lobelii*. A, Habit; B, V. S. through a stroma; C, Asci; D, Ascospores.

Matrix: In culmis emortuis *Lobelia trigona* Roxb. (F. Lobeliaceae). Leg. D. V. Narendra (November 11, 1971), ad Mercara (Coorg, Karnataka), No. AMH 2188 (Holotypus).

Grateful thanks are offered to Prof. M. N. Kamat for his keen interest, to Dr. Emil Muller, Institute of Special Botany, Zurich (Switzerland), for kindly

confirming the identity of the fungus and to the Director, M.A.C.S., Poona, for laboratory facilities.

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### RESISTANCE OF TOMATO CULTIVARS TO THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*

(KOFOID AND WHITE, 1919) CHITWOOD, 1949

ROOT-KNOT nematodes constitute one of the important pests of vegetables in India. Solanaceous crops including tomato suffer from heavy damage when grown in soil infested with *Meloidogyne incognita* and *M. javanica*.

In any nematode control programme, development of nematode resistant varieties constitute an important objective. In addition to chemical and crop rotation methods. Some cultivars and selections have been reported to show resistance to root-knot nematodes by various workers<sup>1-6</sup>.

Five tomato varieties were assessed for their relative resistance to the root-knot nematode, *M. incognita*, at the Experimental Station, Hessarghatta, Indian Institute of Horticultural Research, Bangalore. Cultures of *M. incognita*, maintained on tomato variety, Pusa Ruby, were used as the inoculum source. Seedlings of different varieties raised in nematode free soil (soil treated with D-D at the rate of 60 gallons/acre and stored in wooden boxes for about 6 months) were transplanted in 6" clay pots. At transplantation 100 gm of infested soil (about 5,800 larvae in 100 gm of soil) was added around the root system in each pot. Six replicates were kept for each variety. After 10 weeks the plants were carefully uprooted by breaking the pot and washing the roots under running tap water. The root-knot index was obtained on a scale of 1-5 with the plant showing an index between 1 and 2 rated as resistant (Table I and Fig. 1).

The varieties Pelican, Hawaii-7746, Hawaii-7747 were found resistant to *M. incognita*, with a root-knot index of 1.5, 1.17 and 1.17 respectively, whereas *Lycopersicon pimpinellifolium* and the variety Pusa Ruby with a root-knot index of 4.00 and 5.00 were susceptible.

Dropkin *et al.*<sup>1</sup> have shown that *L. pimpinellifolium* was susceptible to *M. hapla* and *M. incognita acrita*. Khan *et al.* have reported in their

TABLE I

Root-knot index of tomato varieties that show differences in resistance after transplanting in soil infested with *Meloidogyne incognita*

| Variety                              | Root-knot index <sup>a</sup> |
|--------------------------------------|------------------------------|
| Pelican                              | 1.50                         |
| Hawaii-7746                          | 1.17                         |
| Hawaii-7747                          | 1.17                         |
| Pusa Ruby                            | 5.00                         |
| <i>Lycopersicon pimpinellifolium</i> | 4.00                         |

<sup>a</sup> : 1=no galling on roots; 2=1-25% galled; 3=26-50% galled; 4=51-75% galled and 5=76-100% of roots galled.

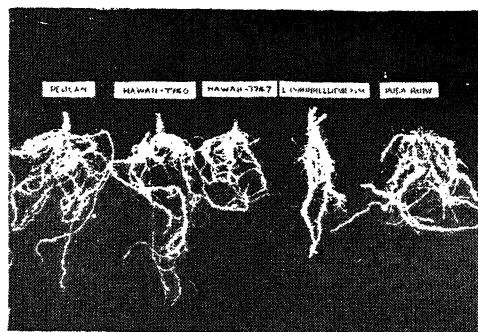


FIG. 1. Roots of different tomato varieties showing different degrees of infestation : Pelican, Hawaii-7746, 7747—Resistant. *L. pimpinellifolium* and Pusa Ruby—susceptible.

studies that *L. pimpinellifolium* to be resistant to *M. incognita* while here it has been recorded as susceptible. It is quite possible that there may be a variation in the species. *L. pimpinellifolium* or there may be existence of different strains in *M. incognita*. Sikora *et al.*<sup>5</sup> reported a similar observation with respect to screening of root-knot nematode resistant tomato cultivars against *M. javanica*. They observed that two resistant varieties VFN-8 and VFN-368 were found heavily galled to a Pant Nagar population. This shows that the known resistant varieties should be carefully screened against the local populations of root-knot nematodes before they are recommended for a particular area for cultivation or before their inclusion in a breeding programme.

The authors are grateful to Dr. G. S. Randhawa, Director, for his encouragement to carry out this work. Thanks are also due to Dr. V. G. Prasad

for going through the manuscript and to Dr. M. V. B. Rao for the supply of Hawaiian lines.

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#### EMBRYOLOGICAL STUDIES IN *MEINECKIA* *PARVIFOLIA* (WIGHT) WEBSTER

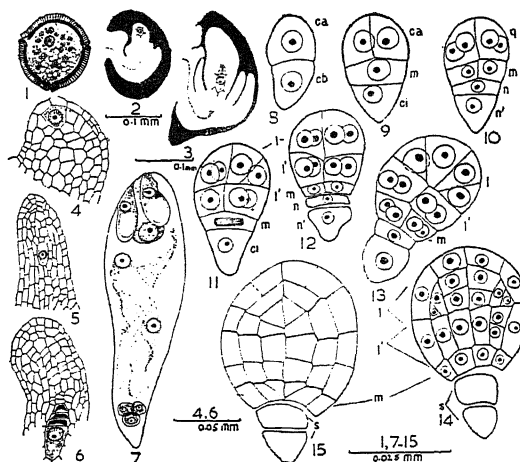
THE genus *Meineckia* Baill. of the Euphorbiaceae, belongs to the tribe Phyllanthae of the subfamily Phyllanthoideae. Webster<sup>1</sup> recognised 19 species in the genus and drew attention to the extraordinary confusion in the taxonomic history of the genus in that the various species were earlier included in six different genera: *Cluytiandra*, *Flueggea*, *Neopeltandra*, *Peltandra*, *Phyllanthus* and *Securinega*. Of the 19 species recognised, four species are represented in India and Ceylon and *Meineckia parvifolia* is one of them. This species of somewhat infrequent occurrence in South India was treated by Gamble<sup>2</sup> as *Neopeltandra suberosa* which in its turn was synonymous with *Phyllanthus suberosus* Muell. Arg. Gamble erected the genus *Neopeltandra* to be distinct from *Phyllanthus* based on the number of stamens which is 5 in *Neopeltandra* and 3 in *Phyllanthus*. However, Webster took into consideration a combination of vegetative, floral, capsular and seed characters (especially the occurrence of a pistillode in the male flowers and the structure of the seed) merged it with the genus *Meineckia*. So far, there is no information on the embryology for any species of this interesting genus.

In *Meineckia parvifolia*, the male archesporium is formed by a single row of hypodermal cells. The wall of the quadrisporangiate anther consists of an epidermis, fibrous endothecium, two middle layers and a secretory type of tapetum with a single layer of binucleate cells. The divisions of the pollen mother cells are simultaneous and cytokinesis takes place by furrowing and tetrahedral type of

pollen tetrads are formed. The pollen grains are shed at the 2-celled stage (Fig. 1) and according to Punt<sup>3</sup> they resemble those in various unspecialized genera of the Phyllanthae. Kohler<sup>4</sup> made no mention about the pollen grains in this genus.

The ovary is superior, tricarpeal syncarpous and trilocular with a pair of collateral, bitegmic anatropous, crassinucellate ovules in each loculus on axile placentation. An obturator is developed from the placental region<sup>5</sup> (Figs. 2, 3). The female archesporium is single celled (Fig. 4) and cuts off a parietal cell and the resultant megaspore mother cell becomes deep seated (Fig. 5). The embryo sac development is of the Polygonum type (Figs. 6, 7). These features are similar to those of investigated species of the genus *Phyllanthus*<sup>6-8</sup>.

The endosperm is of the Nuclear type. The first division of the zygote is transverse giving rise to a 2-celled proembryo (Fig. 8). The apical cell *ca* divides vertically and the basal cell *cb* transversely and thus the 4-celled proembryo at the end of the II cell generation has its four cells arranged in three tiers (Fig. 9) as in *Phyllanthus grandifolius*<sup>9</sup> and unlike in other species of *Phyllanthus* studied<sup>6-8</sup>. A quadrant *q* is formed in the tier *ca* (Fig. 10). Transverse divisions in each cell of the quadrant differentiate an octant comprising two tiers *l* and *l'* of four cells each (Figs. 11, 12). The middle cell *m* undergoes a vertical division and behaves as the hypophyseal initial (Figs. 10-15). The cell *ci* undergoes a transverse division and forms a bicelled suspensor (Figs. 12-15). The derivatives of the



FIGS. 1-15. Fig. 1. 2-celled pollen grain. Figs. 2, 3. Long sections of ovules at the mmc and linear tetrad stage, respectively. Figs. 4, 5, 6. Long sections of part of ovules showing stages in the megasporogenesis. Fig. 7. Mature embryo sac. Figs. 8-15. Stages in the embryo development.

tier 1 contribute to the stem tip and cotyledons, those of 1' to hypocotyl and radicle, and those of *m* to the root tip and root cap. Hence the embryogeny falls under the Period I, Series A, Megarchetype IV according to Souèges (see Crété<sup>10</sup>) or keys out to the Euphorbia variation of the Onagrad type according to Johansen<sup>11</sup>.

There is no significant embryological data for species of the genera, *Chytandra*, *Flueggea*, *Neopeltandra*, *Peltandra*, and *Securinega* with which *Meineckia* was formerly associated excepting 5 species of *Phyllanthus* (this genus has 480 species). Comparative embryological approach, hence tends to be inconclusive. Webster, however, singled out the seed characters as the best, diagnostic feature for the genus. Further embryological work on species of *Meineckia* is in progress to find the implications of embryology, correlated with data on other grounds, in evaluating the status of the genus.

I wish to express my sincere thanks and gratitude to Prof. J. Venkateswarlu for guidance and encouragement. I am grateful to Prof. Grady L. Webster, of the University of California, USA, for providing me with valuable literature and useful correspondence on this topic. My thanks are due to Mr. D. Sarveswara Rao for help in preparing this paper.

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#### OCCURRENCE OF AECIAL AND PYCNIAL STAGES OF *PUCCINIA HELIANTHI* SCHW. IN INDIA

SUNFLOWER rust, caused by *Puccinia helianthi* Schw., has been reported from Bombay on *Helianthus annuus*<sup>1</sup>. But in Coimbatore, though this rust has been observed very early in the twenties itself no published report of its occurrence appears to have been made<sup>2</sup>. Siddiqui<sup>3</sup> noted only the uredial

and telial stages of this rust on the variety "Sunrise" at Coimbatore.

Though this rust has been reported to be autoecious as early as 1934 by Arthur<sup>4</sup>, the aecial and pycnial stages do not appear to be of common occurrence. Perisic<sup>5</sup> reported aecial and pycnial stages from Yugoslavia. No record of the occurrence in India of these two stages of the rust is available. During the last week of August 1974, the aecial and pycnial stages of this rust were observed in the campus of the Tamil Nadu Agricultural University on the leaves of 85 day's old plants of the sunflower variety EC 68413 heavily infected by *P. helianthi*, along with the uredial stage.

The pycnia are amphigenous, aecia hypophyllous cupulate and aeciospores globoid, wall colourless and minutely verrucose (Fig. 1). This appears to



FIG. 1. Transverse section of Sunflower leaf showing aecia and pycnia.

be the first record of the aecial and pycnial stages of the rust in India. Studies on the life-cycle and host range of this rust are in progress.

Our grateful thanks are due to Dr. T. K. Kandasamy, Head of the Department of Plant Pathology, for providing all facilities and encouragement.

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## SHORT SCIENTIFIC NOTES

### A New Indicator for Direct EDTA Titration of Zinc Ions

The present note describes the use of diphenyl carbazide as indicator for the direct EDTA titration of zinc ions. The stock solutions of zinc sulphate, EDTA and indicator were 0.1 M, 0.01 M and 1.0% respectively. Solutions of interfering ions were 0.2 M.

Diphenyl carbazide gives a pink colour with zinc ions. There is a sharp colour change on titration with EDTA from pink to pale yellow at the end point. When an aliquot of  $Zn^{2+}$  solution is titrated with EDTA, zinc can be estimated accurately in the pH range 2.0–3.0 with the help of the indicator investigated. Titrations, carried out with different concentrations of the indicator, showed that an addition of 2 drops of 1.0% solution of the indicator gave satisfactory results for 5–10 ml of 0.01 M zinc sulphate solution. The titration could be satisfactorily carried out in the temperature range 10–60° C. At 1.5 mg concentration of zinc, 50 times excess of  $Na^+$ ,  $K^+$ ,  $NH_4^+$ ,  $Cr^{3+}$ ,  $NO_3^-$ ,  $SO_4^{2-}$ , 5 times excess of  $Ni^{2+}$ ,  $Co^{2+}$ ,  $Mg^{2+}$ ,  $Mn^{2+}$  and 2 times excess of  $Ca^{2+}$ ,  $Sr^{2+}$ ,  $Ba^{2+}$ ,  $Cl^-$  and  $Cu^{2+}$  could be tolerated. Similar observations for other ions and indicators have been reported<sup>1-3</sup>.

Take 5.0 ml aliquot of 0.01 M zinc sulphate solution, adjust pH 2.0–3.0 using 1–2 ml solution of acetate-HCl buffer of pH 2.0, add 2 drops of 1.0% solution of the indicator and titrate against 0.01 M solution of EDTA to pale yellow colour.

5.0 ml aliquots of 0.01 M zinc sulphate were analysed according to the suggested procedure. The standard deviation was 1.0%.

Thanks are due to the Principal, Borsad Science and Law College and E.M.H.S. Trust, Borsad, for extending laboratory facilities.

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### A Gravimetric Method for Estimating the Root-Knot Incidence

The root-knot nematode, *Meloidogyne* spp., has a polyphagous nature in attacking almost all the economically important crops. The root-knot formation has a great significance in the disease development and thus, may prove to be an important parameter in estimating the disease incidence. The incidence of the disease caused by this nematode has been rated variously. Smith<sup>1</sup> merely counted the number of galls in the infected plants and prepared an index based on the percentage of galls, to express the disease development. More or less similar methods, based on visual observations, were used to express the severity of disease, by Jones and Nirula<sup>2</sup>, Khan *et al.*<sup>3</sup> and Mishra and Prasad<sup>4</sup>, without taking into account the dimensions or the mass of the galls, although the severity of the disease depends on the mass rather than the number of galls on the roots.

In the proposed method, the disease incidence is expressed in terms of knot-root ratio, based on the fresh weight of the whole root system of nematode-infected plants and that of the root portions transformed into knots.

*Procedure.*—The inoculated/infected plants were uprooted and washed thoroughly to remove the adhering soil particles. The water particles, adhering to the roots due to washing, were removed using blotting sheets. The roots were then examined for the presence of root-knots. The whole root system was weighed. The visible root-knots of varying size (from minute to large size) were removed under a stereoscopic dissecting microscope, by cutting the knotted portions. The isolated knotted portions of the roots were weighed. The weight of the infected tissue (knots) per g of root was calculated. The results were expressed as the mean values of randomized replicates, in terms of knot-root ratio.

The present quantitative technique is most simple and exact particularly for root-knot, and further, it yields quick results with the least efforts, as it does not involve any cumbersome measurements of the individual root or knot, nor does it need any histological analysis to find out the different stages of nematode development.

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### Effect of Climate on the Variation in Electro-Chemical Properties of Natural Humic Acids

Natural humic acid isolated from (1) Dehradun forest soil (pH 5.6), (2) Ooty non-forest acid soil (pH 4.7), (3) Bangalore red soil (pH 4.9) and (4) Nagpur black soil (pH 7.2) was subjected to potentiometric titration. The Nagpur soil humic acid showed greater buffer capacity upto pH 7, indicating abundance of COOH groups while the Ooty soil humic acid showed strong buffering beyond pH 8 showing the presence of phenolic OH groups. Humic acid from Dehradun and Bangalore soils, however, showed little variation in buffer capacity between different pH ranges (pH 4-7, 7-8 and 8-9). This suggests that the reactions, parent phenol  $\rightarrow$  polyphenol  $\rightarrow$  carboxyl, are occurring in these soils. Thus, the results indicate that the variations in the oxidation status of humic acid are largely dependent on the climatic conditions under which soil formation takes place.

TABLE I  
Alkali required to titrate humic acids upto different pH levels

| Place     | Amount of alkali required<br>(me. per 100 gm humic acid) |            |            |
|-----------|--|------------|------------|
|           | pH 4.0-7.0   | pH 7.0-8.0 | pH 8.0-9.0 |
| Nagpur    | 27.3   | 9.6        | ..         |
| Dehradun  | 10.6   | 7.3        | 9.0        |
| Ooty      | 6.0  | 3.0        | 12.6       |
| Bangalore | 5.6  | 4.3        | 5.0        |

Author is grateful to Dr. B. Ramamoorthy, formerly Head of the Division, for his kind interest in the work.

Division of Soil Science and M. B. SEN GUPTA.  
Agricultural Chemistry,  
IARI, New Delhi-12, July 2, 1974.

### A New Bacterial Disease on "Varalaxmi" a Hybrid Cotton

"Varalaxmi" cotton, a hybrid (*Gossypium hirsutum* ♀  $\times$  *Gossypium barbadense* ♂) was reported to be suffering from wilt disease. When such samples were examined they invariably showed vascular discolouration either in the form of disjointed streaks or continuous streaks extending

from root region upto the stem. In some of the samples received later, there was hardly any discolouration in the vascular tissue of the stem and taproot, but it was noticed only in secondary and tertiary roots. Transverse sections of such roots did not show any vascular mycelium. From the samples examined, it appeared that plants of any age group are liable for infection.

Isolations from such discoloured vascular tissues, invariably, yielded a bacterial culture on Nutrient agar. Culture obtained from tissue isolation was further purified by dilution-plating, and culture from single well-isolated colony was transferred to sterile distilled water and maintained at 20°C for further studies. This culture was successfully used in artificial reproduction of the disease, by inoculating the injured side roots of 10 days old cotton seedlings, grown in sterile soil.

The bacterium makes dull white, slimy growth on NA. Bacterium is a short rod gram negative, non-acid fast, non-spore forming with a single polar flagellum. Bacterium does not hydrolyse starch, does not produce indole and H<sub>2</sub>S. It does not produce fluorescent pigment on fluorescein medium<sup>1</sup> when observed under ultraviolet light, and makes fluidal opaque irregularly circular colonies, with slight pink colour at the centre on Triphenyl tetrazolium chloride medium<sup>2</sup>.

Based on the morphological, cultural and biochemical characters, the organism under study has been identified as *Pseudomonas solanacearum* E.F.Sm., a new record on cotton. Studies are in progress regarding its relationship with other isolates of *P. solanacearum* from Potato, Tomato and Eggplant.

The authors are grateful to Dr. H. C. Govindu, Senior Professor, UNDP, for providing facilities. Dept. of Plant Pathology, V. V. SULLADMATH. Univ. of Agril. Sciences, R. K. HEGDE. Bangalore 560024, B. G. PATIL KULKARNI. February 12, 1975. P. C. HIREMATH.

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### Effect of Crowding on the Nymphal Duration of *Hieroglyphus nigrореpletus* Bol. (Orthoptera: Acrididae)\*

*Hieroglyphus nigrореpletus* Bol. (Phadka grasshopper) is a pest of rice, millet, sugarcane and other crops in India. This pest is of considerable importance in the Tarai regions of Uttar Pradesh because of their abundance in this part of the country. The present writers consider it as 'monsoon enemy of man' because they appear just after the

first monsoon showers in their breeding areas and start feeding voraciously on the seedlings of the crops and chop them off. Roonwal (1945) has studied the seasonal history of this pest at Banaras (Varanasi) but much attention has not been paid to the effect of crowding on the nymphal duration of this pest. Norris (1952) could not find any significant difference in the nymphal duration of *Schistocerca gregaria* Forskal when reared under isolated as well as crowded conditions, but the present writers have recorded a marked effect of crowding on the nymphal duration of *H. nigro-repletus* Bol. The nymphs of *H. nigrorepletus*, when reared collectively, completed their development in a shorter period of time as compared to those reared individually. This behaviour is of vital importance to this pest as it retains a solitary nature throughout its life-cycle.

The experiment on the crowding effect on nymphal duration was carried out with fifty freshly hatched nymphs which were reared at  $32^{\circ}\text{C} \pm 2^{\circ}\text{C}$  in each glass jar ( $15 \times 10$  cm). At the same temperature only ten hoppers were reared in a glass jar of the same size. Obviously the former batch of hoppers got less space in terms of per hopper space as compared to the latter batch of hoppers. Nymphs reared in crowded conditions took 35 to 45 days; but those reared under isolated conditions took 75.5 to 80.5 days in completing their development. The average nymphal duration under crowded and isolated condition has, therefore, been recorded as 40.0 and 80.0 days respectively.

Thanks are due to Prof. M. Shafi, D.A.P. and Prof. Reyat Khan, for their interest and encouragement.

Department of Zoology, S. KAMAL A. RIZVI.  
Aligarh Muslim University, SHAMSHAD ALI.  
Aligarh (India), SHALENDRA K. YADAV.  
October 11, 1974. SAMI UDDIN KHAN.

\* Presented at the annual meeting of the National Academy of Sciences, Allahabad, held at Jodhpur in October, 1973.

1. Norris, M. J., *Anti-Locust Bull.*, London, 1952, 13, 1.
2. Roonwal, M. L., *Bull. Ent. Res. London*, 1945, 36 (3), 339.

#### Reduction in Oil Content of Yellow Mosaic Infected Soybean Seeds

Like other plants soybean is afflicted with a number of virus diseases which cause disturbances in the host physiology. In the present study, the

yellow mosaic disease of soybean, possibly caused by Mung bean yellow mosaic virus, was found to reduce the oil content of seeds in all the varieties tested.

Six varieties of soybean, viz., Bragg, Clark-63, Lee, Amsoy, Picket and Local-2 were selected for oil estimation. For each variety two lots each of 20 seedlings were taken. One lot was treated with white flies (*Bemisia tabaci* Gennadius) fed on diseased soybean leaves. The other lot was left healthy. The plants were kept in insect proof conditions. When plants attained maturity their seeds were collected separately and their oil content estimated, using Soxhlet's method. The data are presented in Table I.

TABLE I  
Oil content in healthy and yellow mosaic infected seeds of different varieties of soybean (data based on oil extracted from 4g seeds under each treatment)

| Varieties | Per cent oil content |          |
|-----------|----------------------|----------|
|           | Healthy              | Diseased |
| Bragg     | 25.3                 | 17.6     |
| Clark-63  | 26.1                 | 17.4     |
| Lee       | 25.5                 | 14.3     |
| Amsoy     | 25.6                 | 16.6     |
| Picket    | 25.8                 | 16.0     |
| Local-2   | 21.4                 | 11.8     |

The seeds from diseased plants contained less oil than the seeds from healthy plants. The varieties relatively more susceptible to the disease showed more marked reduction in oil content. Lee and Local-2 had maximum reduction while Bragg had least.

Harris *et al.*<sup>1</sup> reported that soybean infected with chlorotic mottle virus (soybean strain) had a reduced oil percentage in the seeds. Demski *et al.*<sup>2</sup> reported that tobacco ring spot virus infection reduced the palmitic, linoleic and linolenic acid proportions of the oil while stearic and oleic acids increased in soybean plants (Cv. Lee).

The author is thankful to Prof. K. S. Bhargava for providing necessary laboratory and library facilities.

Botany Research Laboratory, B. D. SUTERI,  
University of Gorakhpur,  
Gorakhpur (U.P.), October 9, 1974.

1. Harris, H. B., Jellum, M. W. and Kuhn, C. W., *J. Agric. Food Chem.*, 1970, 18, 911.
2. Demski, J. W., Harris, H. B. and Jellum, M. W., *Phytopathology*, 1971, 61, 308.

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## REVIEWS AND NOTICES OF BOOKS

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**Modern Microscopy—Elementary Theory and Practice.** By C. F. A. Culling. (Butterworths and Co., Ltd., 88, Kingsway, London, WC 2 B 6AB). 1974. Pp. xii + 148. Price £ 1.50.

This book on *Modern Microscopy* by C. F. A. Culling (Butterworth and Co., Publishers Ltd., 1974) deals with theoretical principles and practical aspects of a wide range of microscopes.

There are, in addition to the introduction, 12 chapters. The first chapter exclusively deals with the various optical and mechanical aspects of a compound microscope, the illumination, setting up the microscope, and finally the micrometry. An additional feature of this chapter is the treatment of lenses and their aberrations which is an asset to every microscopist. In the second chapter the author briefly describes and recommends the use of the comparison microscope for comparing by direct observation of the two specimens instead of relying on human memory for this comparison. The brief third and fourth chapters deal respectively with the low-power dissecting microscope and the dark-ground microscope. The next two chapters, namely the fifth and sixth, introduce the concepts of fluorescence and discuss the fluorescent microscope techniques. Autofluorescence, fluorescent staining and fluorescent antibody techniques are treated rather elaborately. In the seventh chapter the author treats some aspects of polarisation as needed for the use of a polarising microscope. The next chapter deals with the phase contrast microscope. In chapters nine and ten the interference contrast microscope (Nomarsky) and the interference microscope are well presented. Brief and succinct in the presentation, these two chapters deliver the necessary information on the fundamental principles, setting up and using the microscopes.

While dealing with photomicrography in chapter eleven, the author has considered at length on some essential prerequisites for good image such as elimination of vibration, use of camera with and without lens, light sources, magnification and resolution and use of colour filters to obtain contrast. Electron microscopy forms the subject-matter of the last chapter. A brief theory and construction of an electron microscope is followed by details on the techniques of preparation of tissue sections for examination with the microscope. Staining methods and preparation of electron microscope grids are also included.

This booklet is an asset to the libraries and students. Elementary theory and principles involved in each microscope, methods of preparing materials, and staining techniques have been presented in a lucid and detailed manner. As the author claims, this book truly meets the needs of students and research workers alike. The book is recommended to students of biology, chemistry, physics, pathology, and laboratory technologists.

M. R. RAO.

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**Annual Review of Biophysics and Bioengineering (Vol. 3).** Edited by L. J. Mullins, William A. Hagins, Lubert Stryer and Carol Newton. (Annual Reviews, Inc., 4139 El Camino Way, Palo Alto, California 94306), 1974. Pp. 401. Price: U.S.A. \$ 12.00; Foreign \$ 12.50.

The volume contains a number of reviews on various different branches of biophysics and bioengineering, and the scope of the volume is best illustrated by giving the titles of the different articles, which are the following: Kinetics of allosteric Enzymes; Some Applications of Calorimetry in Biochemistry and Biology; Structure of Photoreceptor Membranes; Protein Model Refinement Based on X-Ray Data; The Properties of Water in Biological Systems; Conformational Changes in DNA Molecules; The Structure and Spectra of the Chromophore of the Visual Pigments; Rotational and Translational Diffusion in Membranes; Thermodynamic Relationships in Mitochondrial Oxidative Phosphorylation; Collagen as a Biomaterial; Automata and Biology; The Analysis of Convection and Diffusion in Capillary Beds; Freezing Injury and its Prevention in Living Cells; and Scintillation Scanning of the Brain. The two articles of special interest to the reviewer were the one by L. H. Jensen on protein structural refinement and the one by Stenzel, Miyata and Albert L. Rubin on collagen as a biomaterial.

Jensen's article describes the refinement of the crystal structures of a number of proteins using X-ray data and standard techniques of Fourier and least-squares refinement. It is interesting to note that in the case of Rubredoxin, the R-value came down from nearly 0.4 to 0.126. As a result, several of the side-group atoms came out very prominently and could be readily located. However, in the case of cytochrome *c*, the protein molecule itself did not refine, but only the solvent

molecules could be located. The method is, therefore, of great value in small proteins and has still to be worked out in the case of larger protein molecules.

The chapter on collagen as a biomaterial starts with the biochemical and biophysical properties of collagen, in which the primary structure, cross links and the action of collagenase on collagen are treated. This is followed by an account of the biological properties of collagen and the clinical applications of various types of collagenase material such as collagen heterografts, reconstituted collagen, extruded collagen fibres, collagen membranes and films, and collagen gels and sponges. The article is brief, but contains a valuable collection of information to the bioengineer for using collagen for "eye surgery, skin and bone replacement, blood vessel and heart valve implantation, dialysis and drug delivery and possibly result in the restructuring of normal tissues".

The volume as a whole is highly informative and useful, and is strongly recommended to every biological library.

G. N. RAMACHANDRAN.

**The Red Shift Controversy.** By George B. Field, Halton Arp and John R. Bahcall. (W. A. Benjamin Inc., Advanced Book Programme, Reading, Massachusetts, U.S.A.), 1973. Pp. xvi + 324. Cloth \$19.50; Paper \$11.00.

The Frontiers in Physics Series concerns itself with the problem of communicating in a coherent fashion the recent exciting developments in the active new fields of physics. In this task, the present book has succeeded admirably. It presents a quick survey of the problems raised by the large Doppler shifts and the large apparent luminosities of quasi stellar objects (quasars) and their cosmological implications.

Since Hubble's observation in 1929 of a linear relationship between the distance of a far-off galaxy and the Doppler red shift interpreted as a velocity of recession from us, the model of an expanding universe has been firmly accepted with the Hubble's law as the observational evidence. The discovery of quasars in the early sixties has thrown up a problem. If their large red shifts are interpreted to mean that they are at very great distances, then their high apparent luminosity implies an unusually large intrinsic luminosity and energy content for the quasar. On the other hand if an acceptably large intrinsic luminosity is assumed and the apparent luminosity used to infer the distance of the quasar from us, then there is a

breakdown of the Hubble's law. This present debate in the field of cosmological studies is summarized in the book, with Dr. Arp stating the case for the breakdown of the Hubble's law for the quasars, Dr. Bahcall stating the case for the unusual luminosity of the quasars and Dr. Field acting as the moderator.

The book is based on the discussion held at the American Association for Advancement of Science in December 1972. The book has been released in May 1974. Apart from the survey articles of Arp and Bahcall, the book includes reprints of about 30 significant papers published on the subject. Inevitably newer observations will render any book out of date, particularly a book of this type. However there is no doubt that, like another astronomical phenomenon namely the meteor, the book will illumine the field for a while and fade away from view afterwards, having fulfilled its destined role.

P. S. NARAYANAN.

## ANNOUNCEMENTS

### Award of Research Degrees

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Mathematics to Shri Umakanta Mohapatra for his thesis entitled "Flow and Heat Transfer in Visco-elastic Liquids".

The M.S. University of Baroda has awarded the Ph.D. degree in Biochemistry to Shri Shashikant Jagannath Tarwadi for his thesis entitled "Studies on Algal Bacterial Symbiosis in High Rate Oxidation Ponds using *Oscillatoria* Spp."; Ph.D. degree in Biochemistry to Shri Chandrakant Mohanlal Upadhyay for his thesis entitled "Effects of Dietary Variations on Bone Composition in Rats"; Ph.D. degree in Psychology to Shri Prasant Kumar Gangopadhyay for his thesis entitled "Social Intelligence and Its Relationship with Abstract and Mechanical Intelligence".

### Books Received

*Radiometric Methods of Exploration.* By V. L. S. Bhimasankaram. (Centre of Exploration Geophysics, Osmania University, Hyderabad 500 007), 1974. Pp. xvi + 212. Price Rs. 20.00; \$4.00 (postage extra).

*Indian Journal of Earth Sciences* (Vol. 1, No. 1). (Indian Society of Earth Sciences, Department of Geology, Presidency College, Calcutta 700012), 1974. Pp. ix + 130. Price: Annual Subscription: India, Rs. 40.00; U.S., \$10.00; £4.00; Personal Subscription in India: Rs. 20.00.

## INFORMATION TO CONTRIBUTORS

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# MIXED LIGAND COMPLEXES OF COBALT (III) WITH N, N'-ETHYLENEDIAMINE-BIS (SALICYLIDENEIMINE) AND BIDENTATE MONOBASIC NO AND OO DONOR LIGANDS

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## ABSTRACT

The syntheses of several new cobalt (III) mixed ligand complexes containing dibasic ONNO donor quadridentate ligand salen [where salen H., = N, N'-ethylenediaminebis (salicylideneimine)] and bidentate monobasic NO or OO donor ligands such as quinaldinic acid, N-phenylsalicylaldimine, methylacetoacetate, ethylacetoacetate, and 2-hydroxy-naphthaldehyde are described. The complexes are characterised by conductance measurements, electronic spectra, infrared spectra, magnetic susceptibility and molecular weight measurements. The complexes are green in colour, monomers, diamagnetic and non-electrolytes. In these mixed ligand complexes salen adopts an unusual nonplanar twisted configuration and the bidentate ligand occupies two *cis* positions. The electronic spectral and magnetic data indicate distorted octahedral nature of the complexes. The electronic spectra of the complexes in chloroform and pyridine are quite different. In pyridine the complexes undergo rearrangement and two pyridine molecules enter the coordination sphere with simultaneous removal of the bidentate ligand.

## INTRODUCTION

THE syntheses and single crystal X-ray structure determination of cobalt (III) heterochelates containing nonplanar quadridentate ligand salen and bidentate OO donor ligands  $\beta$ -diketones and salicylaldehyde have been reported<sup>1-4</sup>. We have extended these studies to NO donor ligands. In this communication we report the synthesis and characterisation of such heterochelates containing bidentate NO donor monobasic ligands quinaldinic acid and N-phenylsalicylaldimine. We also report several new heterochelates containing OO donor bidentate monobasic ligands such as methylacetoacetate, ethylacetoacetate and 2-hydroxynaphthaldehyde.

## EXPERIMENTAL

**Materials and Methods.**—Cobalt (II) chloride hexahydrate, salicylaldehyde, ethylenediamine, aniline, pyridine and nitrobenzene were from Sarabhai M. and Co. Methylacetoacetate, ethylacetoacetate, quinaldinic acid and 2-hydroxynaphthaldehyde were obtained from Fluka AG, Switzerland.

The electrolytic conductance measurements were carried out with a Radelkis type OK 102/1 conductivity bridge and a dip type of cell. The magnetic susceptibility measurements were carried out at room temperature by the Gouy method using  $\text{Hg}[\text{Co}(\text{NCS})_4]$  as a calibrant. Infrared spectra were recorded in KBr pellets on a Perkin Elmer Model 21 infrared spectrophotometer. Each spectrum was calibrated with a polystyrene film. Electronic spectra were obtained with the Beckman DK2 recording spectrophotometer using 10 mm matched quartz cells. Molecular weights were determined by the freezing point method in nitrobenzene.

N-phenylsalicylaldimine was prepared by refluxing salicylaldehyde and aniline in ethanol for half an hour and subsequent cooling. Co(salen) was prepared according to the method of Diehl and Hach<sup>5</sup>. This was oxidised to the corresponding cobalt (III) complex,  $[\text{Co}(\text{salen})]_2\text{O}_2$  by passing air through a chloroform suspension of Co(salen) for 3 hr.

Preparation of  $[\text{Co}(\text{quinaldinic acid})(\text{salen})]\text{H}_2\text{O}$ : Quinaldinic acid (0.53 g) was dissolved in 15 ml of absolute alcohol. This was added to a suspension of Co (salen) (1 g, 0.003 mole) in 20 ml ethanol. The mixture was heated on a water bath for 1 hr and filtered after cooling to room temperature. The residue was dissolved in minimum amount of 1 : 1 ethanol : chloroform mixture, filtered and the filtrate was partially evaporated on a water bath. The separated green precipitates were suction filtered, washed with alcohol followed by ether and dried under vacuum at room temperature. Yield = 20%. This complex cannot be obtained in pure form starting from  $[\text{Co}(\text{salen})]_2\text{O}_2$ .

All other complexes were prepared by following the general procedure described below:

$[\text{Co}(\text{salen})]_2\text{O}_2$  (1 g) was suspended in 20 ml of absolute alcohol. To this an ethanolic solution of the appropriate ligand (0.0030 mole in 15–20 ml) was added and the mixture was heated on a water bath for 1 hr and cooled to room temperature. In the case of methylacetoacetate and ethylacetoacetate, the mixture was not heated but kept at room temperature for 2 hr with occasional stirring. This was filtered and the residue was treated similarly as described under the quinaldinic acid complex. In the case of methylacetoacetate and ethylacetoacetate the partial evaporation of solvents was done under a fan at room temperature. The yield varied from 25–40%.



TABLE I  
Analytical and molecular weight data of cobalt (III) complexes

| Complex  | Stoichiometry   | Analytical data |      |      |       |       |     |     |      | Molecular weight |        |       |
|--|---|-----------------|------|------|-------|-------|-----|-----|------|------------------|--------|-------|
|  |   | Calcd.          |      |      |       | Found |     |     |      | Medium           | Calcd. | Found |
|  |   | %C              | %H   | %N   | %Co   | %C    | %H  | %N  | %Co  |                  |        |       |
| [Co (quinaldinic acid) (salen)]·H <sub>2</sub> O | CoC <sub>28</sub> H <sub>22</sub> N <sub>3</sub> O <sub>3</sub> | 60.58           | 4.27 | 8.16 | 11.46 | 61.4  | 4.7 | 8.3 | 11.4 | —                | —      | —     |
| [Co (N-phenylsalicylaldimine) (salen)]           | CoC <sub>29</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> | 66.79           | 4.61 | 8.06 | 11.32 | 64.3  | 5.0 | 8.3 | 11.4 | Nitro benzene    | 521    | 600   |
| [Co (methylacetoacetate) (salen)]                | CoC <sub>21</sub> H <sub>21</sub> N <sub>2</sub> O <sub>5</sub> | 57.27           | 4.77 | 6.36 | 13.41 | 56.4  | 5.1 | 6.5 | 13.6 | do               | 454    | 482   |
| [Co (ethylacetoacetate) (salen)]                 | CoC <sub>22</sub> H <sub>23</sub> N <sub>2</sub> O <sub>5</sub> | 58.15           | 5.07 | 6.17 | 12.99 | 57.0  | 5.9 | 6.4 | 12.5 | —                | —      | —     |
| [Co (hydroxynaphthaldehyde) (salen)]             | CoC <sub>17</sub> H <sub>21</sub> N <sub>2</sub> O <sub>3</sub> | 65.32           | 4.23 | 5.65 | 11.90 | 63.4  | 4.5 | 5.9 | 11.9 | Nitro benzene    | 496    | 488   |

### RESULTS AND DISCUSSION

The bidentate mono basic ligands react with [Co(salen)]<sub>2</sub>O<sub>3</sub> in ethanol medium at the temperature of steam bath. Methylacetoacetate and ethylacetoacetate react with [Co(salen)]<sub>2</sub>O<sub>3</sub> in ethanol even at room temperature. Apparently the complexes were formed by breaking the  $\mu$  oxygen bridge in [Co(salen)]<sub>2</sub>O<sub>3</sub> in the presence of the bidentate ligand. Our attempt to prepare heterochelate complex with NO donor ligand picolinic acid by the reaction of this ligand with [Co(salen)]<sub>2</sub>O<sub>3</sub> in ethanol was unsuccessful and we isolated the red cobalt picolinate as the major product. But in the case of other bidentate ligands we did not notice the formation of homochelates and the desired heterochelates were the major products. The complexes are non-electrolytes ( $\Lambda_{\text{m}} = 1.6\text{--}2.7 \text{ ohm}^{-1}\text{cm}^2 \text{ mole}^{-1}$ ) in methanol. The molecular weights in the nitrobenzene indicate that the complexes are monomer in this solvent (see Table I). The magnetic susceptibility measurements indicate that the complexes are diamagnetic as expected for d<sup>6</sup> cobalt (III) complexes of octahedral symmetry.

The  $\nu(\text{OH})$  stretch of the ligand salenH<sub>2</sub> at 2600 cm<sup>-1</sup> (ref. 6) is absent in the complexes indicating coordination through the phenolic oxygen atoms. But the heterochelate complex with quinaldinic acid has a medium intense  $\nu(\text{OH})$  band at 3300 cm<sup>-1</sup> in accordance with the elemental analysis of the complex. This water molecule in [Co (quinaldinic acid) (salen)] H<sub>2</sub>O is the water of crystallisation, as the coordinated water usually exhibit  $\nu(\text{OH})$  band around 3100 cm<sup>-1</sup> (ref. 7). In some of our complexes the carbonyl stretching

vibrations occur at lower frequencies in comparison to those of the free ligands. This lowering of the  $\nu(\text{C=O})$  bands is indicative of the oxygen coordination of the bidentate ligands. The  $\nu(\text{C=N})$  and the conjugated phenyl ring vibrations of salenH<sub>2</sub> shifts to higher frequencies in the complexes and this observation is in line with the infrared spectra of other metal complexes of salenH<sub>2</sub><sup>6</sup>.

We have recorded the electronic spectra of the complexes in chloroform and pyridine. The complexes exhibit three bands at around 16,000, 25,000 and 39,000 cm<sup>-1</sup> (see Table II). The first band

TABLE II  
Electronic spectral data of cobalt (III) heterochelates

| Complex  | Medium     | $\nu_{\text{max}}$ (log $\epsilon$ )<br>cm <sup>-1</sup> |
|--|------------|--|
| [Co (quinaldinic acid) (salen)]·H <sub>2</sub> O | Chloroform | 16670 (2.52),<br>26310 (3.77),<br>39220 (4.71)           |
|  |            | 26200 (4.71)   |
|  | Pyridine   | 26200 (4.71)   |
| [Co (N-phenylsalicylaldimine) (salen)]           | Chloroform | 16560 (2.45),<br>25000 (3.96),<br>38460 (4.88)           |
|  |            | 24900 (3.97)   |
|  | Pyridine   | 24900 (3.97)   |
| [Co (methylacetoacetate) (salen)]                | Chloroform | 16390 (2.68),<br>25500 (3.80),<br>39220 (4.71)           |
|  |            | 25400 (3.84)   |
|  | Pyridine   | 25400 (3.84)   |
| [Co (ethylacetoacetate) (salen)]                 | Chloroform | 16450 (2.68),<br>25500 (3.80),<br>39220 (4.69)           |
|  |            | 25400 (3.82)   |
|  | Pyridine   | 25400 (3.82)   |
| [Co (hydroxynaphthaldehyde) (salen)]             | Chloroform | 16470 (2.54),<br>27670 (3.87),<br>38020 (4.76)           |
|  |            | 25800 (4.03)   |
|  | Pyridine   | 25800 (4.03)   |

at  $\sim 16,000\text{ cm}^{-1}$  is assigned to the  ${}^1A_{1g} \rightarrow {}^1T_{1g}$  transition and the second band at  $\sim 25000\text{ cm}^{-1}$  is assigned to the  ${}^1A_{1g} \rightarrow {}^1T_{2g}$  transition coupled with the metal-ligand transition. The third band at  $\sim 39,000\text{ cm}^{-1}$  is assigned to the transitions in the ligand and/or charge transfer transitions. We have noticed that the electronic spectra of the complexes in chloroform and pyridine differ appreciably. The band at  $\sim 16,000\text{ cm}^{-1}$  observed in chloroform solution is missing in the electronic spectra of the complexes in pyridine. It seems that this band is moved to higher energy and is covered underneath the intense transitions. The cause of this marked difference in the electronic spectra is attributed to the pyridine coordination with the simultaneous removal of the bidentate ligand from the coordination zone<sup>4</sup>.

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## EFFECT OF SUNLIGHT ON THE VIABILITY AND CHROMOGENESIS OF THREE WIDESPREAD BACTERIA

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#### ABSTRACT

Viability and chromogenesis of *S. marcescens*, *P. aeruginosa* and *S. aureus* in water, milk, and soil have been studied over a period of 180 days. Viability in darkness and diffused light has been shown to be longer than under sunlight. The effect of sunlight, diffused light, and darkness on the colour of the pigment produced by these bacteria has been reported.

#### INTRODUCTION

FROM the previous study on the viability of *Serratia marcescens* in 5 g aliquots of soil inoculated with approximately 50 million cells, it was concluded that the organism could not survive in the air-dry state of the soil under the influence of direct sunlight for 30 days, though it did survive under the semi-saturated and water-logged states of soil for the same period of exposure. Under diffused light and in darkness, however, the organism survived for 30 days in the dry soil, although a discernible difference was observed in its morphology and pigmentation on nutrient agar. On the other hand, samples of soil with the organism incubated under darkness gave good growth of the organism on nutrient agar even in the dry state and luxuriant growth as well as normal pigmentation were witnessed from the semisaturated and the water-logged samples of soil.

*Pseudomonas aeruginosa* inoculated (about  $50 \times 10^6$ ) in the dry soil<sup>2</sup>, unlike *S. marcescens*, could survive 30 days exposure to direct sunlight; the exposure, however, affected its pigmentation both under the direct light and diffused light. Incubation of soil under darkness did not have such effect especially under humid conditions which favourably influenced both viability and pigmentation in that the semi-saturated soil kept under diffused light yielded fairly well pigmented growth whereas the water-logged soil gave both normal growth and characteristic pigmentation.

Like *Pseudomonas aeruginosa*, *Staphylococcus citreus* also survived 30 days exposure to all light-weather conditions, but presented variable picture of growth and pigmentation<sup>3</sup>. It was in this context that viability and pigmentation of the two rod-shaped chromogenic bacteria and a strain of *Staphylococcus aureus* (instead of *S. citreus* used

in the earlier study) were considered of interest for longer periods of exposure under the different light-weather conditions in the three media, water, milk and soil. The results of a prolonged study, extending over 6 months, are presented in this paper.

#### MATERIALS, METHODS AND RESULTS

Cultures of *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* used in the experiments were those derived from the stock maintained in the Microbiology and Pharmacology Laboratory for nearly two decades. The cultures were first tested for their purity, pigmentation in particular, and other characteristics in general. Pigmentation-wise, the three bacteria were respectively dark red (Hue 10 R 3/6), dark olive green (Hue 5 Y 3/2) and yellow (Hue 5 Y 8/6) as per Munsell Soil Color Charts<sup>4</sup>.

Skimmed milk, obtained from the whole milk supplied in bulk by the Hessaraghatta dairy farm, water from the tap (Bangalore City supply) and soil from the reddish garden loam soil (unsieved, pH 6.9) were the other materials used, in the experiment after sterilization in the autoclaves. The sterility of these materials was tested by the routine sterility tests before their inoculation with the three cultures.

The liquid media were distributed in 25 ml aliquots in nine 100 ml Erlenmeyer flasks and in 10 ml quantities in nine test tubes (6 × 5/8") and the soil in 50 g lots and 20 g lots in flasks and test tubes respectively. Into flasks was inoculated 1 ml of young liquid cultures (3 flasks and 3 test tubes for each culture) and in tubes the inoculum was

and incubated at the three environmental conditions for observation of growth and pigmentation. Similar tests were performed in water and soil with the three bacteria mentioned in the text. The experimental set-up would become clear from the scheme.

#### Scheme followed for testing viability and pigmentation

Milk: Culture. *S. marcescens* (Subcultured on N. agar in all cases)

| Exposed to | Incubated in sunlight (L) |
|------------|---------------------------|
| sunlight   | diffused light (DL)       |
|            | darkness (D)              |
| Exposed to | sunlight (L)              |
| diffused   | diffused light (DL)       |
| light      | darkness (D)              |
| Kept in    | sunlight (L)              |
| darkness   | diffused light (DL)       |
|            | darkness (D)              |

Viability was recorded by general appearance of growth on the agar slant and no particular attention was paid to ascertaining the extent of growth. Pigmentation was noted and, where possible, the colour was compared to "Munsell colour Chart" and the colour recorded. Table I presents the viability in days and Table II the colours of the three organisms on nutrient agar after incubation thereof in the three media under the different light weather conditions. The pigmentation Hues reported in Table II correspond to those recorded for cultures which survived the incubation period in days as shown in Table I.

TABLE I  
Viability in days in milk, water and soil

| Incubation<br>Organisms | Milk |    |     | Water |    |    | Soil |    |     |
|-------------------------|------|----|-----|-------|----|----|------|----|-----|
|                         | L    | DL | D   | L     | DL | D  | L    | DL | D   |
| Viability in days       |      |    |     |       |    |    |      |    |     |
| <i>P. aeruginosa</i>    | ..   | 10 | 120 | 120   | 10 | 90 | 120  | 30 | 180 |
| <i>S. aureus</i>        | ..   | 10 | 120 | 120   | 10 | 90 | 120  | 30 | 180 |
| <i>S. marcescens</i>    | ..   | 10 | 120 | 120   | 10 | 90 | 120  | 30 | 180 |

L = Sunlight.

DL = Diffused light.

D = Darkness.

pipetted in 0.1 ml aliquots. The contents were well mixed and one flask and one test tube of each of the three natural media were incubated respectively in direct sunlight, in diffused light and in a completely darkened room.

At intervals, a loopful (small quantity) of the inoculated soil, milk or water was inoculated on 9 slants of nutrient agar for each of the culture

It will be clear from the results that *S. marcescens* not only survived 180 days in soil under both darkness and diffused light but retained its original Hue 10 R 3/6, deemed as dark red. The organism lived however for only 120 days in milk under diffused light and darkness as it did in water only in darkness. It is extremely interesting to note that this organism continued to synthesise its pigment

TABLE II

*Exact colours of the three organisms on nutrient agar for different periods of incubation  
(nutrient agar after growth, 48 hr)*

| From milk to N. agar slants   |                                  |                                  |                                  |
|-------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Organisms                     | L<br>(10 days)                   | DL<br>(120 days)                 | D<br>(120 days)                  |
| <i>Serratia marcescens</i>    | HUE 10R 6/6<br>(Light red)       | HUE 10R 4/6<br>(Red)             | HUE 10R 4/6<br>(Red)             |
| <i>Pseudomonas aeruginosa</i> | HUE 5Y 6/2<br>(Pale olive green) | HUE 5Y 3/2<br>(Dark olive green) | HUE 5Y 3/2<br>(Dark olive green) |
| <i>Staphylococcus aureus</i>  | HUE 5Y 8/4<br>(Pale yellow)      | HUE 5Y 8/6<br>(Yellow)           | HUE 5Y 8/6<br>(Yellow)           |

| From water to N. agar slants  |                                  |                            |                            |
|-------------------------------|----------------------------------|----------------------------|----------------------------|
| Organisms                     | L<br>(10 days)                   | DL<br>(90 days)            | D<br>(120 days)            |
| <i>Serratia marcescens</i>    | HUE 10R 6/6<br>(Light red)       | HUE 10R 4/6<br>(Red)       | HUE 10R 3/6<br>(Dark red)  |
| <i>Pseudomonas aeruginosa</i> | HUE 5Y 6/2<br>(Pale olive green) | HUE 5Y 3/2<br>(Dark olive) | HUE 5Y 3/2<br>(Dark olive) |
| <i>Staphylococcus aureus</i>  | HUE 5Y 8/4<br>(Pale yellow)      | HUE 5Y 8/6<br>(Yellow)     | HUE 5Y 8/6<br>(Yellow)     |

| From soil to N. agar slants   |                                  |                            |                            |
|-------------------------------|----------------------------------|----------------------------|----------------------------|
| Organisms                     | L<br>(30 days)                   | DL<br>(180 days)           | D<br>(180 days)            |
| <i>Serratia marcescens</i>    | HUE 10R 3/4<br>(Light dusty red) | HUE 10R 3/6<br>(Dark red)  | HUE 10R 3/6<br>(Dark red)  |
| <i>Pseudomonas aeruginosa</i> | HUE 5Y 6/2<br>(Pale olive green) | HUE 5Y 3/2<br>(Dark olive) | HUE 5Y 3/2<br>(Dark olive) |
| <i>Staphylococcus aureus</i>  | HUE 5Y 8/4<br>(Pale yellow)      | HUE 5Y 8/6<br>(Yellow)     | HUE 5Y 8/6<br>(Yellow)     |

of original Hue in water for 120 days, whereas the Hue got changed to red (10R 4/6) in milk kept under diffused light and darkness. This red colour was retained in water kept in DL, though the organism survived under this condition for only 90 days.

In direct sunlight this organism survived in damp soil for as many as 30 days, an observation made for another strain long time back<sup>1</sup>. In both milk

and water, under direct light, the organism however could not live longer than 10 days.

Both *P. aeruginosa* and *S. aureus*, like *S. marcescens*, survived for 10 days only in sunlight when suspended in water or milk. In other respects also their viability was similar to that shown by *S. marcescens*. However, *P. aeruginosa* seems to retain its pigmentation property better in that it continued to elaborate its dark olive (5Y 3/2) in milk under

darkness, and in water and soil under both darkness and diffused light. Likewise, *S. aureus* displayed the ability to retain its pigmentation property in all media under darkness and diffused light only: under direct sunlight, it changed from yellow to pale yellow.

The viability and pigmentation studies on the three bacteria thus reveal the extent to which they can resist the sterilising effect of sunlight and explains their widespread distribution throughout the universe.

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### IN VITRO INFLUENCE OF SCORPION VENOM ON THE ENZYME SYSTEMS OF SCORPION, *HETEROMETRUS FULVIPES*

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#### ABSTRACT

Influence of scorpion venom on the enzyme systems of its own tissues was studied. Aminotransferases and aldolase activities were unaffected in the hepatopancreas. Similar results excepting a decrease in alanine aminotransferase in neuronal mass was noticed. Both the nonspecific acid and neutral protease activities were enhanced by the addition of venom in hepatopancreas and neuronal mass. The results are discussed in terms of ammonia production and ammonogenic coma during venom toxicity.

#### INTRODUCTION

THE cannibalistic behaviour in the scorpions, reported earlier<sup>1</sup>, prompted us to study the biochemical changes in the scorpion tissues during the paralysed state of the animal when the venom from the opponent is injected into it. In the present study we have selected representative enzymes from glycolytic pathway, protein and amino acid metabolism.

#### MATERIALS AND METHODS

Scorpions were collected from local hilly terrain and were adapted to the laboratory conditions. They were kept in separate glass jars to prevent cannibalism amongst them and were fed daily with cockroaches.

Venom was collected from scorpions as reported earlier<sup>2</sup> excepting that distilled water was used instead of K-Na phosphate buffer. The protein content of the venom was adjusted to 500 µgm/ml.

Hepatopancreas and the cephalothoracic nerve mass along with ventral nerve cord (referred hitherto as neuronal mass) were isolated from the scorpions. Tissue homogenates (10% for hepatopancreas and 5% for neuronal mass) were prepared either in 0.25 M sucrose solution or in distilled water at 4° C and were centrifuged at 2,000 rpm for 15 min. The supernatants were collected for enzyme assay.

Aspartate aminotransferase (L-Aspartate: 2-α-oxoglutarate aminotransferase, E.C.2.6.1.1.) and alanine aminotransferase (DL-Alanine: 2-α-oxoglutarate aminotransferase E.C.2.6.1.2.) were assayed<sup>3</sup>. Aldolase activity was determined by the method of Burns and Bergmeyer<sup>4</sup>. Non-specific acid and neutral proteases were assayed<sup>5</sup>.

The experimental tubes contained 0.2 ml of venom corresponding to 100 µgm of protein whereas the control tubes contained the same volume of distilled water. All the protein determinations were made by the method of Lowry *et al.* (1951)<sup>6</sup>. Each enzyme was assayed in six different animals and in duplicates and the results were expressed as mean of these values.

#### RESULTS AND DISCUSSION

The scorpion venom seems to induce differential effects on the enzyme activity levels in its hepatopancreas and neuronal mass (Table I). The activity levels of aldolase are comparatively less altered than the other enzymes on the addition of venom suggesting that glycolysis as represented by hexose cleavage is least sensitive in both the tissues studied. The venom of *Buthus minax* was known to induce hyperglycemia which was believed to be due to elevated glycogenolysis or gluconeogenesis<sup>7</sup>. Further, the venom of *Centruroides sculpturatus* is known to act at acetyl CoA level<sup>8</sup>. Thus the venom

effects do not involve glycolysis. The venom of *H. fulvipes* was found to inhibit succinate dehydrogenase activity in its neuronal mass<sup>2</sup> and decreased the respiratory rate in cockroach<sup>9</sup> indicating that one of the targets of the venom action is the operation of the citric acid cycle retaining the usual glycolytic rate so as to meet the energy demands.

Though the venom has been shown to be devoid of proteolytic activity<sup>10</sup>, it has elevated the acid and neutral protease activity levels in both the hepatopancreas and neuronal mass (Table I) probably

Thus the venom effects are at the aerobic segment of the carbohydrate metabolism as represented by lowered succinate dehydrogenase activity. The lowered metabolism of keto acids in citric acid cycle and elevated glutamate dehydrogenase indicate the possibility of ammonia toxicity.

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TABLE I  
 Effect of scorpion venom on enzymes of scorpion, *Heterometrus fulvipes*

|  | Hepatopancreas  |                  |                | Neuronal mass  |                 |                |
|--|-----------------|------------------|----------------|----------------|-----------------|----------------|
|  | Control         | Venom            | % over control | Control        | Venom           | % over control |
| Aspartate amino transferase <sup>1</sup> | 1.74<br>± 0.41  | 1.47<br>± 0.46   | - 15<br>N.S.   | 0.72<br>± 0.03 | 0.67<br>0.0     | - 6<br>N.S.    |
| Alanine amino transferase <sup>2</sup>   | 2.99<br>± 0.46  | 2.44<br>± 0.52   | - 18<br>N.S.   | 0.45<br>± 0.06 | 0.22<br>± 0.03  | - 51<br>N.S.   |
| Aldolase <sup>3</sup>                    | 86<br>± 29.43   | 95<br>± 29       | 10<br>N.S.     | 122<br>± 4     | 122<br>± 4      | 0              |
| Protease <sup>4</sup><br>(5.0 pH)        | 10.42<br>± 3.71 | 28.91<br>± 4.99  | 177<br>p 0.02  | 2.78<br>± 0.17 | 3.67<br>± 0.32  | 27<br>p 0.05   |
| Protease <sup>1</sup><br>(7.0 pH)        | 19.30<br>± 6.91 | 49.40<br>± 19.12 | 155<br>p 0.05  | 2.23<br>± 0.26 | 10.30<br>± 0.16 | 397<br>p 0.001 |

1 and 2 Activity expressed as  $\mu$  moles of pyruvate/mg protein/hr.  
 3 do.  $\mu$  moles of FDP hydrolysed/mg protein/hr.  
 4 do.  $\mu$  moles of tyrosine/mg protein/hr.  
 N.S., Not significant.

increasing the amino acid level. The oxidative deamination of amino acids are considered to be liberating ammonia since the glutamate dehydrogenase activity was reported to increase<sup>2</sup>. Since aminotransferase activity levels were also least affected or inhibited by venom (Table I) the accumulated ammonia may result in decreased neuronal activity either directly or indirectly (draining out the keto acid metabolites of the citric acid cycle) thus restricting the energy generation.

The greater reduction of alanine aminotransferase activity in the neuronal mass in comparison with the hepatopancreas by the venom indicated lower pyruvate feeding from the amino acid source into the citric acid cycle. Unlike the alanine aminotransferase, the aspartate aminotransferase activity level was less affected by the venom (Table I) in both the tissues suggesting that the feeding of oxaloacetate or  $\alpha$ -ketoglutarate is unaltered during venom toxicity.

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# RADIOCARBON DATES OF NEOLITHIC-CHALCOLITHIC SAMPLES

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**W**E report below the  $^{14}\text{C}$  dates from the neolithic sites of Bihar, Karnataka and some Harappan and allied sites of Rajasthan, Punjab and Gujarat. Experimental procedures are given very briefly here, for details see Kusumgar *et al.*, 1963; Agrawal *et al.*, 1971, Agrawal and Kusumgar, 1973.

Extraneous matter in the form of rootlets and sticking soil, etc., were removed manually. For the removal of humic contamination, alkali pretreatment was given to all the samples except TF-576, -748 and -699. Acid leaching was used to get rid of soil carbonates, if any. Samples were counted in the form of methane gas in gas proportional counters. NBS oxalic acid was used as modern reference standard.

For each sample two B.P. dates are given. The first is based on the half-life value of 5568 yrs and the second, within parenthesis, is based on 5730 yrs value. The same half-life based dates should be used for all intercomparisons. A.D. 1950 should be used as reference year for conversion of B.P. dates to A.D./B.C. scale.

## GENERAL COMMENT ON DATES

It is interesting to note that Bara is not a pre-Harappan site but even later than Kalibangan in point of time. This shows that some of the so-called pre-Harappan cultures were in fact rural cultures which continued without much change even after the end of the Harappa Culture. The antiquity of Chirand now extends back to c. 1700 B.C., though some neolithic backwaters of Bihar continued upto the I millennium B.C. Hallur dates (TF-570 and -576), if confirmed from other sites, may extend the antiquity of iron in the south to the beginning of the I millennium B.C.

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## $^{14}\text{C}$ DATES WITH SAMPLE DESCRIPTIONS

### Bara, Punjab, India

Bara (Lat.  $30^{\circ} 56' \text{ N.}$ , Long.  $76^{\circ} 32' \text{ E.}$ ) is situated in the Rupar District of Punjab. Bara excavations were conducted by Dr. Y. D. Sharma. Samples submitted by Director General of Archaeology (DGA), New Delhi. *Comment*:  $^{14}\text{C}$  dates assign a post-Harappan period to Bara Culture.

TF-1204, Bara Culture,  $3690 \pm 150$   
(3795  $\pm$  155)

Wood charcoal from Bara 6, Pit 2 sealed by Layer 3, depth 0.8 m., Field No. Bara-6/2/1971. *Comment*: trench is on the slope of the mound.

TF-1205, Bara Culture,  $3730 \pm 95$   
(3840  $\pm$  95)

Wood charcoal from Bara 5, Layer 9, depth 2.2 m, Field No. Bara-5/3/1971.

TF-1206, Bara Culture,  $3135 \pm 100$   
(3230  $\pm$  100)

Wood charcoal from Bara 5, Layer 10, depth 2.5 m, Field No. Bara-5/4/1971.

TF-1207, Bara Culture,  $3490 \pm 85$   
(3595  $\pm$  90)

Wood charcoal from Bara 7, Pit 1 sealed by Layer 1, depth 1.2 to 1.4 m, Field No. Bara-7/5/1971.

### Barudih, Bihar, India

Barudih (Lat.  $22^{\circ} 45' \text{ N.}$ , Long.  $85^{\circ} 57' \text{ E.}$ ), District Singhbhum, is a Neolithic Culture site. Samples submitted by Dr. A. K. Ghosh, Calcutta University, Calcutta. *Comment*: Site appears to be a backwater of Neolithic Culture of Bihar.

TF-1099, Neolithic Culture,  $2625 \pm 105$   
(2700  $\pm$  110)

Carbonaceous clay from Locus N-S 0.68 m. E-W 0.45 m, depth 1.74 m, sample No. 2 (Acc. No. 141).

TF-1100, Neolithic Culture,  $2920 \pm 200$   
(3005  $\pm$  210)

Carbonaceous clay from Locus N-S 29.5 to 56 cm, E-W 12.5 to 37.5 cm, depth 91.3 cm, sample No. 3 (Acc. No. 144).

TF-1101, Neolithic Culture,  $2475 \pm 85$   
(2545  $\pm$  90)

Carbonaceous clay from Locus N-S 38 cm, E-W 59.5 cm, depth 104 to 114 cm, sample No. 4 (Acc. No. 152).

TF-1102, Neolithic Culture,  $2540 \pm 90$   
(2610  $\pm$  90)

Carbonaceous clay from Locus N-S 31.5 to 57 cm, E.W. 62.0 to 98.5 cm, depth 94.3 cm, sample No. 5 (Acc. No. 147).

### Chirand, Bihar, India

Chirand (Lat.  $25^{\circ} 45' \text{ N.}$ , Long.  $84^{\circ} 45' \text{ E.}$ ), District Saran, is a Black-and-Red Ware site. The site was excavated by Dr. B. P. Sinha, Patna University, Patna, who submitted the samples. *Comment*:  $^{14}\text{C}$  dates push the age of Neolithic-Chalcolithic period at Chirand back to c. 1700 B.C. Samples below layer 14 show mix-up, perhaps due to subsidence.

TF-1028, Black-and-Red Ware, 3390  $\pm$  90  
(3490  $\pm$  90)

Charcoal from Trench CRD-XI, Layer 10, depth 6.5 m.

TF-1029, Black-and-Red Ware, 2915  $\pm$  85  
(3000  $\pm$  90)

Charcoal from Trench CRD-XI, Layer 10, depth 6.5 m.

TF-1030, Black-and-Red Ware, 3430  $\pm$  100  
(3530  $\pm$  100)

Charcoal from Trench CRD-XI, Layer 11, depth 6.9 m.

TF-1031, Neolithic Culture, 3525  $\pm$  135  
(3625  $\pm$  140)

Charcoal from Trench CRD-XI, Layer 12, depth 7.2 m.

TF-1032, Neolithic Culture, 3600  $\pm$  150  
(3705  $\pm$  155)

Charcoal from Trench CRD-XI, Layer 13, depth 7.5 m.

TF-1033, Neolithic Culture, 3390  $\pm$  110  
(3490  $\pm$  110)

Charcoal from Trench CRD-XI, Layer 14, depth 8.5 m.

TF-1034, Neolithic Culture, 3420  $\pm$  110  
(3520  $\pm$  115)

Charcoal from Trench CRD-XI, Layer 15, depth 9 m.

TF-1035, Neolithic Culture, 3125  $\pm$  100  
(3220  $\pm$  105)

Charcoal from Trench CRD-XI, Layer 16, depth 9.25 m.

TF-1036, Neolithic Culture, 2485  $\pm$  120  
(2555  $\pm$  125)

Charcoal from Trench CRD-XI, Layer 17, depth 10.1 m.

TF-1125, Neolithic Culture, 3365  $\pm$  150  
(3465  $\pm$  155)

Charcoal from Trench CRD-XI (Ext.), Layer 14, depth 7.5 m.

TF-1126, Neolithic Culture, (?) 2290  $\pm$  120  
(2355  $\pm$  125)

Charcoal from Trench CRD-XI (Ext.), Layer 15, depth 8.15 m.

TF-1127, Neolithic Culture, 3230  $\pm$  95  
(3325  $\pm$  100)

Charcoal from CRD-XI, Layer 17, depth 10.3 m.

#### **Hallur, Mysore, India.**

Hallur (Lat. 14° 20' N., Long. 75° 37' E.), District Dharwar, was excavated by Dr. M. S. Nagaraja Rao, Department of Archaeology, Mysore State, who submitted the samples.

TF-570, Neolithic-Megalithic Overlap, 2970  $\pm$  105  
(3055  $\pm$  105)

Charcoal from Trench 1, Layer 4, depth 1.80 to 2.10 m.

TF-576, Neolithic Culture, 3280  $\pm$  105  
(3375  $\pm$  110)

Charcoal from Trench 1, Layer 8, depth 3.60 m.

TF-586, Neolithic Culture, 3055  $\pm$  95  
(3145  $\pm$  100)

Charcoal from Trench 2, pit sealed by Layer 8, depth 2.8 m.

#### **Inamgaon, Maharashtra, India**

Inamgaon (Lat. 18° 35' N., Long. 74° 32' E.), District Poona, a Chalcolithic site, giving a sequence from Malwa to late Jorwe Culture. The site is being excavated by Deccan College, under the direction of Dr. H. D. Sankalia, who submitted the samples.

TF-995, Chalcolithic Culture, 1775  $\pm$  125  
(1825  $\pm$  125)

Charcoal from INM-I, Trench A2, Layer 3.

TF-997, Chalcolithic Culture, 1530  $\pm$  105  
(1575  $\pm$  105)

Charcoal from INM-I, Trench D2, Layer 7, depth 2.8 m.

TF-1235, Chalcolithic Culture, 3135  $\pm$  90  
(3225  $\pm$  95)

Charcoal from INM-I, Trench C6, Layer 3 depth 0.75 m.

TF-1330, Chalcolithic Culture, 3085  $\pm$  100  
(3175  $\pm$  105)

Wood from Trench E7, Layer 4.

#### **Kalibangan, Rajasthan, India**

The twin mounds of Kalibangan (Lat. 29° 25' N., Long. 74° 05' E.), District Sri Ganganagar, are located on the banks of Ghaggar (now dried). Excavations were conducted under the joint supervision of Ss. B. B. Lal and B. K. Thapar. Submitted by the DGA.

TF-160, Harappa Culture, 4060  $\pm$  100  
(4180  $\pm$  105)

Charcoal from Trench XC1, Qd. 2, Layer 12, depth 3.65 m.

TF-942, Harappa Culture, 4055  $\pm$  110  
(4175  $\pm$  115)

Charcoal from Trench XA1, Qd. 4, Layer 12, depth 3.45 m. Rootlets removed.

TF-946, Harappa Culture, 3605  $\pm$  100  
(3715  $\pm$  105)

Wood charcoal from Trench ZN1, Qd. 1, Layer 7, depth 2.25 m. Rootlets removed.

TF-947, Harappa Culture, 3765  $\pm$  85  
(3875  $\pm$  90)

Wood charcoal from Trench C5, Qd. 3, Layer 34, depth 5.2 m. Rootlets removed.

TF-948, Harappa Culture, 3815  $\pm$  100  
(3930  $\pm$  100)

Charcoal from Trench C5, Qd. 3, Layer 22 depth 3.10 m, Rootlets removed.



**Kodekal, Mysore, India**

TF-748, *Neolithic Culture*, 4285  $\pm$  105  
(4410  $\pm$  105)

Charcoal from Kodekal (Lat. 16° 21' N., Long. 76° 24' E.), District Gulbarga, Trench 2, Layer 4, depth 2 to 3 m, Field No. 948. Sample submitted by Director, Deccan College, Poona.

**Palavoy, Andhra Pradesh, India.**

Palavoy (Lat. 14° 31' N., Long. 77° 09' E.), Anantpur. Sample submitted by Director, Deccan College, Poona.

TF-699, *Ashmound, Modern*

Carbonaceous ash (dung) from Layer 2. Comment: Iron slag was found with the sample.

TF-700, *Neolithic*, 3390  $\pm$  95  
(3490  $\pm$  100)

Carbonaceous ash (dung) from Layer 7, depth 3.0 m, sample No. 2.

TF-701, *Neolithic*, 3805  $\pm$  100 (3915  $\pm$  105)

Charcoal from Layer 9, depth 3.5 m, sample No. 3.

**Surkotada, Gujarat, India.**

Surkotada (Lat. 23° 37' N., Long. 70° 50' E.), District Kutch, was excavated by J. P. Joshi. It revealed a huge fortified Harappan settlement. Sample submitted by the DGA.

TF-1294, *Harappa Culture*, 3620  $\pm$  95  
(3730  $\pm$  100)

Charcoal from Trench XCI, Qd. 3, Layer 3, depth 2.4 to 2.9 m.

TF-1295, *Harappa Culture*, 3780  $\pm$  95  
(3890  $\pm$  100)

Charcoal from Trench G 1, Qd. 3, Layer 8, depth 1.9 to 2.05 m.

TF-1297, *Harappa Culture*, 3635  $\pm$  95  
(3740  $\pm$  95)

Charcoal from Trench C 1, Qd. 4, Pit 1 sealed by Layer 3, depth 2.1 m.

TF-1301, *Harappa Culture*, 3840  $\pm$  130  
(3950  $\pm$  135)

Charcoal from Trench B 1, Qd. 3, Layer 17, depth 5.65 m.

TF-1304 and 1309, *Harappa Culture*, 3645  $\pm$  90  
(3755  $\pm$  90)

Charcoal from Trench ZA 1, Qd. 2, Layer 18 A and 20, depth 6.6 to 7.15 m.

TF-1305, *Harappa Culture*, 3890  $\pm$  95  
(4005  $\pm$  100)

Charcoal from Trench ZA 1, Qd. 2, Layer 19, depth 7.25 m.

TF-1307, *Harappa Culture*, 3510  $\pm$  105  
(3610  $\pm$  110)

Charcoal from Trench XA 4, Qd. 1, Layer 5, depth 1.6 m.

TF-1311, *Harappa Culture*, 3625  $\pm$  90  
(3730  $\pm$  90)

Charcoal from Trench ZA 1, Qd. 2, Layer 4, depth 2.4 m.

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## INTERNATIONAL CONFERENCE ON RAPIDLY QUENCHED METALS

Papers are solicited for the Second International Conference on "Rapidly Quenched Metals" to be held at Massachusetts Institute of Technology (MIT), Cambridge, U.S.A. from November 17 to 19, 1975. The Conference is jointly organized by MIT and Northeastern University and it is anticipated that the proceedings will be published in the form of a book.

The Conference will focus attention on the formation, structure and properties of amorphous and crystalline metastable metals produced by rapid quenching of the liquid deposition from the vapour or deposition from a solution.

A Workshop on "State-of-the-Art and Prospects for Magnetic, Electronic and Mechanical Applications of Amorphous Metals" will be held at Northeastern University, Boston, Massachusetts, U.S.A. from November 20 to 21, 1975.

Those interested in contributing papers should submit an abstract of about 300 words (in duplicate) to Prof. T. R. Anantharaman, Department of Metallurgical Engineering, Banaras Hindu University, Varanasi 221005 by June 1, 1975. The papers must be research papers and must be presented by one of the authors; substitute speakers will not be accepted.

## LETTERS TO THE EDITOR

### A NOTE ON CYLINDRICALLY SYMMETRIC SPACE-TIMES AND PETROV'S CLASSIFICATION

In the invariant theory of gravitational radiation the Riemann curvature tensor plays the central part. Petrov<sup>1</sup> gave classification of vacuum Reimann curvature tensors. Since Weyl's conformal curvature tensor possesses all symmetry properties of the Riemann tensor for non-empty gravitational fields, the algebraic classification of Weyl's conformal curvature tensor can be carried out exactly as in the case of vacuum Riemann tensor. In this paper the Weyl's conformal curvature tensor corresponding to the cylindrically symmetric space-time is classified.

The Einstein-Rosen<sup>2</sup> metric for the cylindrically symmetric space-time may be expressed in the cylindrical polar co-ordinates  $r, \phi, z$  and time  $t$  as

$$ds^2 = e^{2A-2B} (dt^2 - dz^2) - r^2 e^{-2B} d\phi^2 - e^{2B} dr^2 \quad (1)$$

where  $A$  and  $B$  are functions of  $r$  and  $t$  only.

With the help of the expressions for the Riemann curvature tensor and Ricci tensor for metric (1), the non-vanishing components of the Weyl's conformal curvature tensor are

$$\begin{aligned} C_{1212} &= -r^2 e^{-2B} C_{3434} = (m - \sigma) r^2 e^{2A-2B} \\ C_{1224} &= r^2 e^{-2B} C_{1334} = nr^2 e^{2A-2B} \\ C_{2124} &= -r^2 e^{2B} C_{1313} = (m + \sigma) r^2 e^{2A-2B} \\ C_{1114} &= -r^{-2} e^{1A-2B} C_{2223} = 2me^{1A-2B} \end{aligned} \quad (2)$$

where

$$\begin{aligned} m &= e^{2B-2A} \left[ \frac{B_{11}}{6} - \frac{B_{44}}{6} + \frac{A_{44}}{6} - \frac{A_{11}}{6} - \frac{(B_1)^2}{3} \right. \\ &\quad \left. + \frac{(B_1)^2}{3} - \frac{B_1}{3r} \right] \\ \sigma &= e^{2B-2A} \left[ \frac{B_{11}}{2} + \frac{B_{44}}{2} - A_1 B_1 - A_4 B_4 + (B_1)^2 \right. \\ &\quad \left. + (B_1)^2 + \frac{A_1}{2r} \right] \\ n &= e^{2B-2A} \left[ B_{14} + 2B_1 B_4 - A_1 B_1 - A_4 B_4 + \frac{A_1}{2r} \right] \end{aligned} \quad (3)$$

Here and in what follows, the subscripts 1 and 4 after  $A, B$  represent a partial differentiation with respect to  $r$  and  $t$  respectively.

Now following Pirani's<sup>3</sup> scheme, the physical components of the Weyl's conformal curvature tensor given in (2) with the help of the tetrad

$$\lambda_{(a)}^i = \text{diag.} \left( e^{B-A}, \frac{e^B}{r}, e^{-B}, e^{-A} \right) \quad (4)$$

can be written in the form

$$C_{AC} = \\ (A, B = 1, 2, \dots, 6)$$

$$\begin{bmatrix} -2m & 0 & 0 & 0 & 0 & 0 \\ 0 & m + \sigma & 0 & 0 & 0 & n \\ 0 & 0 & m - \sigma & 0 & n & 0 \\ 0 & 0 & 0 & 2m & 0 & 0 \\ 0 & 0 & n & 0 & m - \sigma & 0 \\ 0 & n & 0 & 0 & 0 & -m + \sigma \end{bmatrix} \quad (5)$$

If

$$C_{AB} = \begin{bmatrix} M & N \\ N & -M \end{bmatrix}$$

then

$$P = M + iN \quad \begin{bmatrix} -2m & 0 & 0 \\ 0 & m + \sigma & in \\ 0 & in & m - \sigma \end{bmatrix} \quad (6)$$

The roots of the characteristic equation

$$\begin{aligned} \lambda_1 &= -2m, \quad \lambda_2 = m + \sqrt{\sigma^2 - n^2}, \\ \lambda_3 &= m - \sqrt{\sigma^2 - n^2} \end{aligned} \quad (7)$$

In general the roots  $\lambda_1, \lambda_2, \lambda_3$  are different, and hence according to Synge<sup>4</sup> the canonical form of the Weyl's tensor is of type-I.

Now let us introduce the conditions

$$\sigma^2 - n^2 = 0 \quad \text{and} \quad m \neq 0 \quad (8)$$

Then from (3)

$$\begin{aligned} B_{11} + B_{44} - 2A_1 B_1 - 2A_4 B_4 + 2(B_1)^2 \\ + 2(B_4)^2 + A_{1/r} \\ = \pm (2B_{14} + 4B_1 B_4 - 2A_1 B_1 - 2A_4 B_4 \\ + A_{1/r}) \end{aligned} \quad (9)$$

and

$$B_{11} - B_{44} + A_{11} - A_{44} + 2(B_1)^2 - 2(B_4)^2 - 2B_{1/r} \neq 0 \quad (10)$$

The conditions (9) and (10) remain true if  $A(t \pm r)$  and  $B(t \pm r)$  and in this case the eigen vector corresponding the root  $-2m$  is non-null but the eigen vector corresponding to the repeated root  $m$  is null. Also  $Q = (P - mI) (P + 2mI) \neq 0$ , hence the Weyl's curvature tensor for this space-time is of the type-II.

Further if metric tensor satisfy the conditions

$$\sigma = \pm n \quad \text{and} \quad m = 0 \quad (11)$$

Then all three repeated roots of characteristic equation are equal to zero.

Let  $A = A(t \pm r)$ , then (11) is true if  $B = B(t)$  and

$$\frac{d^2 B}{dt^2} - 2 \left( \frac{dB}{dt} \right)^2 = 0, \text{ i.e.,}$$

$$B(t) = \frac{D}{2} \log(t - C) \quad (12)$$

where  $C$  and  $D$  are arbitrary constants.

Now

$$P = \begin{bmatrix} 0 & 0 & 0 \\ 0 & \sigma & i\sigma \\ 0 & -i\sigma & -\sigma \end{bmatrix} \neq 0, \text{ and } P^2 = 0.$$

There exists only one null eigen vector and all vectors orthogonal to it are also eigen vectors. Hence the Weyl's conformal curvature tensor for this space-time is of type-N.

The author wishes to express his indebtedness to Prof. P. C. Vaidya and Dr. J. Krishna Rao for their help in the preparation of this note.

Department of Mathematics, M. D. PATEL.  
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### NORMAL COORDINATE ANALYSIS OF THE OXYPENTAFLUORIDES OF OSMIUM AND RHENIUM

THE infrared and Raman spectra of  $\text{OsOF}_5$ ,  $\text{ReOF}_5$  and  $\text{IOF}_5$  have been reported by Holloway *et al.*<sup>1</sup>. They have assigned the fundamentals on the basis of a  $C_{4v}$  symmetry. The vibrational spectra and vibrational analysis of  $\text{IOF}_5$  have been reported previously<sup>2</sup>. No such analysis, however, appears to have been carried out for  $\text{OsOF}_5$  and  $\text{ReOF}_5$ . The present work, therefore, was undertaken to extend the normal coordinate treatment to these two molecules. A general valence force field is assumed and the analysis carried out, using Wilson's F-G matrix method<sup>3</sup>.

Molecules with the same structure have been previously studied by various workers<sup>2,4,5</sup>. The set of symmetry coordinates used in the present work is the same as that used by Smith and Begun<sup>2</sup>. The fifteen normal modes of an  $\text{XYZ}_5$  type of a molecule belonging to the  $C_{4v}$  symmetry are classified as  $4a_1 + 2b_1 + 1b_2 + 4e$ , all of which are Raman active while only  $a_1$  and  $e$  are i.r., active. The Raman data reported by Holloway *et al.*<sup>1</sup>, are used in

the present calculations of the force field. The expressions for the symmetrized F and G matrix elements are the same as those reported by Smith and Begun<sup>2</sup>.

The structural parameters<sup>6</sup> used to evaluate the G matrix elements are:  $D = 1.74 \text{ \AA}$ ,  $d_5 = 1.72 \text{ \AA}$ ,  $d = 1.78 \text{ \AA}$ ,  $\angle \alpha = \angle \beta = \angle \gamma = 90^\circ$  for  $\text{OsOF}_5$  and  $D = 1.90 \text{ \AA}$ ,  $d_5 = 1.93 \text{ \AA}$ ,  $d = 1.93 \text{ \AA}$ ,  $\angle \alpha = \angle \beta = \angle \gamma = 90^\circ$  for  $\text{ReOF}_5$ . The force constants were adjusted by successive approximations and the resulting values are reported in Tables I and II

TABLE I

Force constants, and observed and calculated frequencies of  $\text{OsOF}_5$

| Force constants<br>(mdyn/\AA) | Frequencies ( $\text{cm}^{-1}$ ) |       |
|-------------------------------|----------------------------------|-------|
|                               | Obs.                             | Calc. |
| $f_D = 7.904$                 | $a_1$ type                       |       |
| $f_{d5} = 5.440$              | 963                              | 958   |
| $f_d = 4.855$                 | 716                              | 737   |
| $f'_{dd} = -0.143$            | 644                              | 640   |
| $f_{dd} = 0.034$              | 281                              | 261   |
| $f_\gamma = 0.129$            | $b_1$ type                       |       |
| $f_\alpha = 0.261$            | 644                              | 640   |
| $f_\beta = 0.438$             | 210                              | 223   |
| $f'_{aa} = 0.005$             | $b_2$ type                       |       |
| $f'_{\beta\beta} = 0.005$     | 332                              | 332   |
| $f_{\gamma\gamma} = 0.090$    | $e$ type                         |       |
| $f_{a\beta} = 0.070$          | 701                              | 704   |
|                               | 367                              | 367   |
|                               | 263                              | 255   |
|                               | 164                              | 164   |

TABLE II

Force constants, and observed and calculated frequencies of  $\text{ReOF}_5$

| Force constants<br>(mdyn/\AA) | Frequencies ( $\text{cm}^{-1}$ ) |       |
|-------------------------------|----------------------------------|-------|
|                               | Obs.                             | Calc. |
|                               | $a_1$ type                       |       |
| $f_D = 8.358$                 | 990                              | 987   |
| $f_{d5} = 5.498$              | 738                              | 731   |
| $f_d = 4.768$                 | 640                              | 661   |
| $f'_{dd} = 0.058$             | 309                              | 296   |
| $f_{dd} = 0.032$              | $b_1$ type                       |       |
| $f_\gamma = 0.068$            | 652                              | 652   |
| $f_\alpha = 0.399$            | 234                              | 257   |
| $f_\beta = 0.345$             | $b_2$ type                       |       |
| $f'_{aa} = 0.086$             | 334                              | 334   |
| $f'_{\beta\beta} = 0.086$     | $e$ type                         |       |
| $f_{\gamma\gamma} = 0.122$    | 715                              | 717   |
| $f_{a\beta} = 0.204$          | 367                              | 355   |
|                               | 260                              | 275   |
|                               | 125                              | 119   |

along with the observed and calculated frequencies for  $\text{OsOF}_5$  and  $\text{ReOF}_5$  respectively. It can be seen that the axial M-F stretching constant ( $f_{a5}$ ), in both the cases, is greater than the equatorial M-F stretching constant ( $f_a$ ). This result is consistent with those obtained previously<sup>2-4,5</sup> and suggests stronger axial than the equatorial M-F bonding (M=Os, Re). The three stretching force constants— $f_a$ ,  $f_{a4}$  and  $f_5$ —are comparable in magnitudes with the corresponding values reported<sup>2</sup> for  $\text{IOF}_5$  which are 6.99, 4.60 and 4.42 mdyne/Å, respectively.

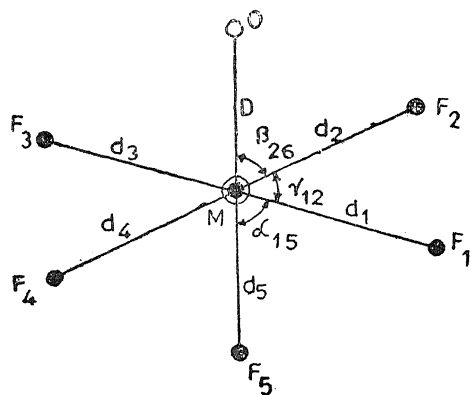


FIG. 1. Internal coordinates of  $\text{MOF}_5$ .

The quantitative estimate of the force field in the case of such molecules of rather unusual structure is of importance for the future vibrational studies of related systems. In fact, based on the results of  $\text{ReOF}_5$  reported here, we have completed the assignment of the vibrational spectrum of  $\text{ReOF}_4$  by a normal coordinate analysis from an incomplete assignment recently reported<sup>7</sup>.

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## ELECTRON SPIN RESONANCE AND OPTICAL SPECTRA OF COPPER (II)-PEPTIDE-AMINO ACID MIXED-LIGAND COMPLEXES

IMPORTANCE of copper ion in biological processes has led in recent years to investigate the naturally occurring systems and model compounds<sup>1</sup>. The activity of biological macromolecules containing copper ion depends upon the selective binding of different functional groups with the metal<sup>2</sup>. For instance enzymatic reactions involving copper occur with the formation of mixed-ligand complexes<sup>3</sup>. Mixed-ligand complexes of copper with amino acids have been isolated from human serum and the complexes appear to play a role in transporting copper through biological membranes<sup>4</sup>. We have undertaken to study the properties of copper (II)-peptide-amino acid mixed-ligand complexes in a systematic way using ESR and visible spectral measurements. This communication presents the results obtained using glycylglycine, glycine and  $\beta$ -alanine as ligands. Simple complexes of these ligands with copper (II) are well studied and the spectral properties are known. Yokoi *et al.* have reported the e.s.r., spectra of several mixed-ligand complexes of copper (II) with amino acids and amines<sup>5</sup>.

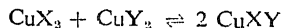
### Experimental

Simple complexes were prepared according to the procedure in the literature. E.s.r. spectra were measured in a Varian E-4 X-band instrument using a  $10^{-2}$  M solution of copper – simple complexes in 1 : 1 water-methanol solution. The spectra of the mixed-ligand complexes were obtained by using a solution of 1 : 1 mixture of the two different simple complexes in 1 : 1 water-methanol.

Visible absorption spectra were scanned under identical conditions at 25° C using a SP 700 A Unicam recording spectrophotometer.

### Results and Discussion

When the copper (II) complexes  $\text{CuX}_2$  and  $\text{CuY}_2$  are mixed together the following equilibrium is established.



with the disproportionation constant given by

$$K_{\text{DXY}} = \frac{[\text{CuXY}]^2}{[\text{CuX}_2][\text{CuY}_2]}$$

From the e.s.r. spectra of the solutions, it could be seen that the line shapes were similar to those observed for single species. If more than one species were present in significant amounts the observed spectrum should have been an admixture of those for the simple complexes. In all the cases studied the line shapes of the spectra were different from the spectra of the simple complexes and the

TABLE I

| Complex                         |    | $\lambda_{\max}$<br>nm | $g_{\parallel}$    | $g_{\perp}$        | $A_{\parallel}$<br>$10^4 \text{ cm}^{-1}$ | $a^2$ |
|---------------------------------|----|------------------------|--------------------|--------------------|---|-------|
| Cu (gly) <sub>2</sub>           | .. | 630                    | 2.231              | 2.055              | 170                                       | 0.547 |
| Cu ( $\beta$ -al6) <sub>2</sub> | .. | 636                    | 2.285              | 2.054              | 129                                       | 0.668 |
| Cu (glygly) <sub>2</sub>        | .. | 640                    | 2.214              | 2.049              | 184                                       | 0.497 |
| Cu (gly) $\beta$ -(ala)         | .. | 637                    | 2.229 <sub>4</sub> | 2.052 <sub>8</sub> | 167                                       | 0.537 |
| Cu (gly) (glygly)               | .. | 636                    | 2.226              | 2.054              | 187                                       | 0.528 |
| Cu ( $\beta$ -ala) (glygly)     | .. | 636                    | 2.215              | 2.052              | 176                                       | 0.502 |

recorded spectra were due to mixed-ligand complexes only. Results published earlier from this laboratory also show that mixed-ligand complexes in general are more stable than the simple complexes<sup>6</sup>.

E.s.r. spectra of the complexes studied show the general pattern characteristic of tetragonal copper (II) complexes with an intense absorption at high field and two or three peaks of less intensity at low fields.  $g_{\parallel}$ ,  $g_{\perp}$  and  $A_{\parallel}$  values were calculated from the low temperature spectra (77° K) and are given in Table I.  $g_{\perp}$  values should be regarded as somewhat approximate since the spectra are not well resolved. At room temperature due to rapid rotations of the molecules anisotropic couplings are averaged out leaving only the isotropic ones, observed spectrum indicates average  $g$  value only.  $g_{\parallel}$  values for the mixed-ligand complexes are found to be in between the  $g_{\parallel}$  values of the simple complexes. In the case of Cu (glygly) ( $\beta$ -ala) complexes although  $g_{\parallel}$  value of the mixed-ligand complex is nearly equal to that of Cu(glygly)<sub>2</sub>,  $A_{\parallel}$  value is in between those observed for the simple complexes. Orbital reduction factors  $a^2$  were calculated using the simplified equation.

$$g_{\parallel} = 2.0023 \left( 1 - \frac{4a^2\lambda}{\Delta} \right)$$

where  $\lambda$  is the spin-orbit coupling constant<sup>7</sup>.  $a^2$  values indicate that the bonding between copper and ligands is covalent in nature with delocalization of electron density into ligand orbitals.

From the e.s.r. data of the naturally occurring copper containing proteins, two types of molecules could be classified, one with considerably higher  $A_{\parallel}$  values than the other type<sup>8</sup>. It is believed that the site symmetry of copper ion in one case is nearly coplanar and in the other distorted tetrahedron<sup>9</sup>. Work is in progress to determine whether copper (II) ion could be forced into a tetrahedron

environment by employing high molecular weight peptides and other ligands.

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#### PIPERIDINIUM AND PYRROLIDINIUM TETRATHIOMOLYBDATES AND TETRATHIOTUNGSTATES

EXTENSIVE work has been carried out<sup>1,2</sup> on alkali metal chalcogenomolybdates and chalcogenotungstates. In continuation of our investigations on thiometallates stabilized by bulky non-spherical counter cations<sup>3,4</sup> studies on piperidinium (pipH) and pyrrolidinium (pyrH) tetrathiomolybdates and

tetrathiotungstates are briefly reported in this paper. The compounds are characterized by u.v., visible and i.r. spectral measurements and thermogravimetric analysis.

Tetrathiomolbdates and tetrathiotungstates of piperidinium and pyrrolidinium were prepared by interacting molybdic oxide or tungstic oxide with about 70% aqueous piperidine or pyrrolidine taken approximately in 1:5 molar ratio. The contents were stirred at 60° with a magnetic stirrer till most of the metal oxide dissolved. The resultant solution was filtered and hydrogen sulphide was passed for 4-6 h. The crystallized tetrathiometalate was filtered, washed with a small amount of acetone and ether and air-dried. The compounds were analyzed for nitrogen, sulphur and metal contents and the analytical results are given in Table I.

The electronic spectra of the compounds taken in water on Beckman DK 2 spectrophotometer gave three characteristic absorption maxima with the molar absorptivity greater than  $10^3$  (Table II). The absorption frequencies are assigned<sup>5</sup> to  $t_1 \rightarrow 2e$  ( $\nu_1$ ),  $t_1 \rightarrow 4t_2$  ( $\nu_2$ ) and  $3t_2 \rightarrow 2e$  ( $\nu_3$ ) electronic transitions according to Viste-Grey M.O. scheme<sup>6</sup>.

Tetrathiomolbdates and tetrathiotungstates exhibit<sup>7</sup> triply degenerate infrared active bands due to M-S vibration in the region 450-500 and 160-180  $\text{cm}^{-1}$ . The present compounds examined on Beckman IR 11 spectrometer gave bands around 460 and 170  $\text{cm}^{-1}$  which are assigned to M-S stretching vibrations.

The thermal decomposition studies of the thiosalts carried out in air and nitrogen atmosphere on Netzsch thermal analyzer indicate that the compounds start decomposing around 120°. The thermal

TABLE I  
Analytical values of tetrathiometalates

| Compound                                | Colour        | Nitrogen (%)                | Sulphur (%)  | Metal (%)    |
|---|---------------|-----------------------------|--------------|--------------|
| (pipH) <sub>2</sub> MoS <sub>4</sub> .. | Red           | Found: 6.81<br>Calcd.: 7.07 | 32.5<br>32.4 | 24.0<br>24.2 |
| (pipH) <sub>2</sub> WS <sub>4</sub> ..  | Golden yellow | Found: 5.63<br>Calcd.: 5.78 | 26.2<br>26.5 | 38.3<br>38.0 |
| (pyrH) <sub>2</sub> MoS <sub>4</sub> .. | Red           | Found: 7.47<br>Calcd.: 7.61 | 33.7<br>34.8 | 26.4<br>26.0 |
| (pyrH) <sub>2</sub> WS <sub>4</sub> ..  | Golden yellow | Found: 5.96<br>Calcd.: 6.14 | 28.4<br>28.1 | 40.1<br>40.3 |

TABLE II  
Spectral data of tetrathiometalates

| Compound                                | Electronic ( $\epsilon \times 10^{-4}$ ) (nm) |            |            | i.r. and far i.r. $\text{cm}^{-1}$ |       |      |
|---|---|------------|------------|------------------------------------|-------|------|
|   | $\nu_1$                                       | $\nu_2$    | $\nu_3$    |                                    |       |      |
| (pipH) <sub>2</sub> MoS <sub>4</sub> .. | 466 (1.12)                                    | 315 (1.81) | 240 (2.86) | 468s                               | 451sh | 180s |
| (pyrH) <sub>2</sub> MoS <sub>4</sub> .. | 466 (1.13)                                    | 316 (1.82) | 242 (2.84) | 478s                               | 462sh | 175s |
| (pipH) <sub>2</sub> WS <sub>4</sub> ..  | 390 (1.82)                                    | 274 (2.71) | 213 (3.51) | 462s                               | 445sh | 173s |
| (pyrH) <sub>2</sub> WS <sub>4</sub> ..  | 392 (1.84)                                    | 275 (2.72) | 215 (3.54) | 460s                               | 450sh | 171s |

The thiometalates are highly soluble in water and precipitate out the respective metal trisulphides on acidification. They are insoluble in ether, ethylacetate, chloroform, carbon tetrachloride, benzene, nitrobenzene, etc. The solids slowly give out hydrogen sulphide and amines with time resulting in the formation of metal trisulphides.

behaviour was found to be similar to that of ammonium tetrathiosalts<sup>8,9</sup>. Around 300° the weight-loss curves indicate the formation of metal trisulphides. The final products of decomposition obtained around 500° on chemical analysis were found to be MoO<sub>3</sub> and WO<sub>3</sub>. When heated in nitrogen atmosphere the final products obtained

around 450° were found on analysis to be metal disulphides. This agrees very well with the weight loss sustained during the decomposition.

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### ESTIMATION OF THIOSEMICARBAZIDE AND ITS METAL COMPLEXES WITH DICHLORAMINE-T

RECENTLY, dichloramine-T (N,N'-dichloro-p-toluene sulphonamide, hereinafter abbreviated to DCT) has been introduced as an oxidimetric titrant by Jacob and Nair<sup>1</sup> in non-aqueous and partially aqueous media and it has been used for oxidizing a variety of compounds<sup>2-4</sup>. Thiosemicarbazide (TSC) is an important compound which is used in the characterization of aldehydes, ketones and polysaccharides and as a metal complexing agent. Chloramine-T has been used as an analytical reagent for estimating TSC and its metal complexes<sup>5-6</sup>. In the present investigations we have examined the behaviour of DCT towards TSC and its metal complexes. It was found that these compounds undergo oxidation with DCT under specified conditions. Hence volumetric methods have been developed for their estimation.

**Materials and Methods.**—Thiosemicarbazide (E' Merck) was purified by recrystallization and an aqueous solution (~0.025 M) was prepared. DCT was prepared and purified by the method of Jacob and Nair<sup>1</sup>. An approximately decinormal solution of the oxidant in glacial acetic acid was prepared and standardized by the iodometric method<sup>1</sup>.

The complexes  $\text{Zn}(\text{TSC})_2\text{SO}_4$ ,  $\text{Zn}(\text{TSC})_2\text{Cl}_2$ ,  $\text{Zn}(\text{TSC})_2(\text{NO}_3)_2$ ,  $\text{Zn}(\text{TSC})_2(\text{ClO}_4)_2$ ,  $\text{Cd}(\text{TSC})_2\text{SO}_4$ ,  $\text{Cd}(\text{TSC})_2\text{Cl}_2$ ,  $\text{Hg}(\text{TSC})_2\text{Cl}_2$ ,  $\text{Ni}(\text{TSC})_2(\text{NO}_3)_2$

and  $\text{Ni}(\text{TSC})_2\text{SO}_4 \cdot 3\text{H}_2\text{O}$  were prepared by methods reported elsewhere<sup>7</sup>.

Detailed investigations of TSC system has brought out the following facts:

(I) TSC forms a complex with DCT which rapidly decomposes with a quick change of colour from orange red to pale yellow and evolution of gaseous products<sup>5</sup>. The complex has a broad absorption band around 350 mμ.

(II) Rate of oxidation is very slow with solutions of TSC in glacial acetic acid, the required stoichiometry being obtained after 16 hours.

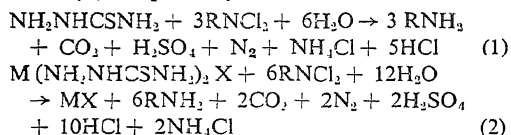
(III) During oxidation, water content of the reaction mixture had to be maintained around 12–15% for the 1:3 stoichiometry to hold good. Slight over oxidations beyond the 12 electron change per mole of TSC were noticed when the water content is less than 12%. This can probably be attributed to the oxidation of  $\text{NH}_4^+$  ion<sup>8</sup>.

(IV) A direct titration with a visual or potentiometric end-point was not practicable.

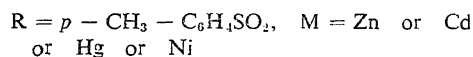
(V)  $\text{N}_2\text{H}_4$ , KCNS and  $\text{PO}_4^{3-}$  ion interfere in the estimation.

(VI) The 1:3 stoichiometry holds only when TSC solutions are added to DCT and not *vice versa*.

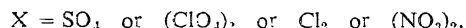
**Recommended Procedure.**—Aliquots of aqueous TSC solution or of the metal complex in pH 4 buffer are added to a measured excess (~50–60%) of 0.1 N DCT maintaining the water content around 12–15% in the reaction mixture. Set aside for 30 minutes (10 minutes in the case of a metal complex), shaking occasionally. Add 10 ml of 20% KI and titrate against 0.1 N sodium thiosulphate. Run a blank with DCT solution alone. The stoichiometry of oxidation of TSC and its metal complexes can be represented by equations (1) and (2) respectively:



Where



and



Some typical results of analyses are shown in Table I and it is noticed that the results of analysis are quantitative within the limits of experimental error.

The percentage error in the estimation of TSC (2–10 mg) was about 0.5. The error was almost negligible (<0.2%) when larger quantities (12–40 mg) of TSC were taken for the estimation.

TABLE I

Estimation of thiosemicarbazide and its metal complexes with Dichloramine-T (% error for values obtained in the neighbourhood of the values indicated in the first column is given)

| Amount of substance estimated | Thiosemi-carbazide | Metal complexes with |                   |                                   |                                    |                   |                   |                   |                                      |                                   |
|-------------------------------|--------------------|----------------------|-------------------|-----------------------------------|------------------------------------|-------------------|-------------------|-------------------|--------------------------------------|-----------------------------------|
|                               |                    | ZnSO <sub>4</sub>    | ZnCl <sub>2</sub> | Zn(NO <sub>3</sub> ) <sub>2</sub> | Zn(ClO <sub>4</sub> ) <sub>2</sub> | CdSO <sub>4</sub> | CdCl <sub>2</sub> | HgCl <sub>2</sub> | NiSO <sub>4</sub> ·3H <sub>2</sub> O | Ni(NO <sub>3</sub> ) <sub>2</sub> |
| 0                             | 0.50               | 0.00                 | 0.42              | 0.50                              | 0.50                               | 0.50              | 0.50              | 0.50              | 0.00                                 | 0.45                              |
| 4                             | 0.50               | 0.20                 | 0.17              | 0.50                              | 0.50                               | 0.00              | 0.50              | 0.50              | 0.00                                 | 0.36                              |
| 8                             | 0.50               | 0.00                 | 0.13              | 0.15                              | 0.30                               | 0.12              | 0.10              | 0.12              | 0.14                                 | 0.36                              |
| 10                            | 0.20               | 0.10                 | 0.17              | 0.18                              | 0.32                               | 0.00              | 0.50              | 0.29              | 0.11                                 | 0.30                              |
| 15                            | 0.10               | 0.15                 | 0.17              | 0.18                              | 0.50                               | 0.50              | 0.30              | 0.00              | 0.15                                 | 0.20                              |
| 20                            | 0.05               | 0.20                 | 0.10              | 0.09                              | 0.00                               | 0.30              | 0.50              | 0.20              | 0.11                                 | 0.00                              |
| 30                            | 0.00               | ..                   | ..                | ..                                | ..                                 | ..                | ..                | ..                | ..                                   | ..                                |
| 40                            | 0.00               | ..                   | ..                | ..                                | ..                                 | ..                | ..                | ..                | ..                                   | ..                                |

Similar results were obtained with various metal complexes, with an error of about 0.5%.

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#### GRAVIMETRIC ESTIMATION OF BERYLLIUM AS BENZIDINE FLUORBERYLLATE

THE usual method of beryllium determination is gravimetric<sup>1</sup> as oxide which, though accurate, is not free from interferences, particularly in presence of aluminium. In the present method beryllium can be estimated in the presence of other ions without much of separation procedure.

In the present method beryllium was precipitated as benzidine fluoroberyllate. Beryllium in solution is first converted into fluoroberyllate by the addition of fluoride ion in excess and then precipitated with benzidine in acetic acid solution. Beryllium in rocks and minerals can easily be converted into fluoroberyllate by fusion with alkali bifluoride and then precipitated as benzidine fluoroberyllate. Benzidine fluoroberyllate<sup>2</sup> like benzidine sulphate is highly soluble in water, flocculent and can easily be filtered using a sintered crucible.

**Procedure.**—Beryllium chloride solution containing 10 mg to 75 mg of beryllium was taken in a 250 ml polythene beaker and diluted to 50 ml. The pH of the solution was adjusted to 3–4 with dil. hydrochloric acid. Sodium fluoride solution was now added taking care to see that the pH of the solution remained at 3–4. The solution was warmed (50°–60° C) and an excess of benzidine (2%) solution in 2 N acetic acid was added along the side of the beaker with constant stirring. It was cooled and filtered through a sintered glass crucible (No. 4), washed with 0.1 M acetic acid, dried in an air oven at 110° C and weighed as benzidine fluoroberyllate. Ten determinations were carried out with varying quantities (20–70 mg) of Be and the mean average error was 0.3%.

**Procedure for Rock and Minerals.**—0.25 gm. of the sample was taken in a platinum crucible and fused with 3–4 times its weight of potassium bifluoride. The fused mass was taken up with water and transferred to a beaker and boiled. If there is a precipitate it should be filtered off. The pH of the solu-



tion was adjusted to 3-4 and then proceeded as stated in the general method.

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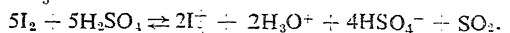
### CHARGE-TRANSFER COMPLEX: INTERACTION OF PENTAATOMIC IODINE CATION WITH DIMETHYLSULFOXIDE

EVER since Mulliken<sup>1</sup> published his work on the electron donor-acceptor systems, a great deal of work has appeared in the literature on the interaction of iodine with electron donors. In this note, we are reporting for the first time, the interaction of iodine pentaatomic cation, which is a more stable species compared to the other iodine poly-cations<sup>2-6</sup>, with the electron donor, dimethylsulfoxide, DMSO, in sulphuric acid.

Since iodine and sulphuric acid are both Lewis acids, no electron exchange should occur when they are mixed. The appearance of iodine band at 503 nm<sup>7</sup>, in 98.1% H<sub>2</sub>SO<sub>4</sub> (Iodine absorbs at 520 nm in *n*-heptane) shows that there is a slight interaction between iodine and sulphuric acid. No other bands could be detected within six hours. The iodine absorption band shows a blue shift in the visible region, as the concentration of sulphuric acid is decreased from 98% to ~20%; below 20%, new bands appear at 290 and 355 nm which are due to I<sub>3</sub><sup>-</sup>.

If the low wavelength absorption of iodine in H<sub>2</sub>SO<sub>4</sub> is due to solute-solvent interaction, the addition of a base solvent, such as water, should affect the absorption and it is indeed observed in the case of water-iodine-sulphuric acid system. At present we are not in a position to suggest any model for such an interaction.

Solutions of iodine in sulphuric acid (> 85% by weight) on standing develop an absorption at 330 nm; we could not detect any band or shoulder around 280 nm in our open cell system<sup>2</sup> (Cary-14R Spectrophotometer). The band at 330 nm is ascribed to the I<sub>5</sub><sup>+</sup> ion which formed by the slow reaction<sup>3</sup>,



When DMSO, an electron donor, is added to a solution containing I<sub>5</sub><sup>+</sup>, the intensity of I<sub>5</sub><sup>+</sup> band (330 nm band) is slightly enhanced and a new band appears at ~350 nm (Fig. 1). The intensity of this new band depends upon the concentration of the DMSO. This behaviour is the typical charac-

teristic of molecular complexes<sup>8,9</sup>. So, the new band can be ascribed as the charge-transfer band which arises due to the interaction of DMSO with I<sub>5</sub><sup>+</sup> in sulphuric acid. The low CT energy of DMSO-I<sub>5</sub><sup>+</sup> (3.54 eV) as compared to that of DMSO-I<sub>2</sub> (4.23-4.58 eV)<sup>10</sup> shows clearly that I<sub>5</sub><sup>+</sup> is a better electron acceptor than iodine molecule itself<sup>8</sup>.

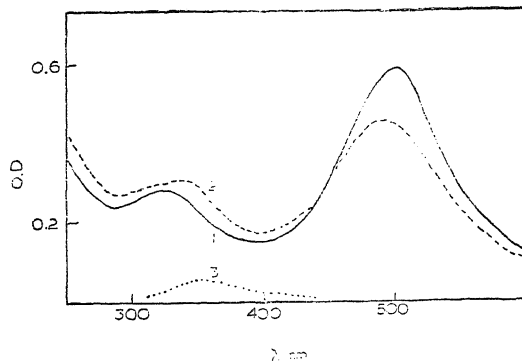


Fig. 1. Absorption spectra of iodine and DMSO in H<sub>2</sub>SO<sub>4</sub>: (1) Iodine in H<sub>2</sub>SO<sub>4</sub> after 72 hours; (2) iodine solution of the same concentration as that of (1) and DMSO; (3) the difference between curves 1 and 3.

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# AMINO ACID COMPOSITION OF EYE LENS OF THE FISH *LABEO BATA* AT DIFFERENT STAGES OF GROWTH

BESIDES the study of the amino acid composition of eye lens of frog, bovine<sup>1</sup> and human being<sup>2</sup>, the changes in protein constituents of eye lens, with the changes in growth, have been studied in rat<sup>3</sup> and human beings<sup>4,5</sup>. No attention has been paid to the study of protein constituent of eye lens in a fish. Therefore to fill the lacuna, the present study is devoted to investigation of the changes in amino acid composition of eye lens of *Labeo bata* at three different stages of growth.

A collection of *Labeo bata* was made from Keetham Lake (Near Agra) and they were grouped according to their length. By dissecting the eyes, the eye lenses of fishes were taken out. Lenses were washed thoroughly with several changes of distilled water, dried in an oven at 100° C and finally ground to fine powder. Total nitrogen in the powder of each group was determined by semi-micro Kjeldhal's method.

Three test samples each equal to about 200 mg of crude protein were hydrolysed with 6 N-HCl under reflux for 24 hours. The hydrolysate was taken in warm water and then filtered. The filtrate was decolorised with a little Darco G-60. After removal of carbon the hydrolysate was evaporated to dryness under reduced pressure and finally dissolved in 10% vol/vol 2-propanol and made up to a definite volume. Tryptophan of the test sample was determined separately by hydrolysis with 10% wt/vol Ba(OH)<sub>2</sub> under reflux for 20 hours.

Complete separation and identification of amino acids were achieved by applying two-dimensional paper chromatography using phenol-water (4:1 wt/wt) as one set and *n*-butanol-acetic acid-water (60:15:25 vol/vol) as another set of solvents.

For estimating individual amino acids chromatograms of a known mixture of amino acids and unknown hydrolysate samples were developed by spraying 0.2% ninhydrin in 2-4% acetic acid in acetone and drying the paper at 60° C for 15 min. Individual coloured spots were cut out from the chromatograms and eluted in 0.42% NaHCO<sub>3</sub> in 48% ethanol and kept undisturbed overnight. The colour intensity of known and unknown samples was read in spectrophotometer.

With the readings of known and unknown samples amino acids in g/100 g of dry eye lens were calculated.

The length and average weight of fish of each group is given in Table I.

Amino acids and total nitrogen of eye lens of fish *Labeo bata* are given in Table II.

TABLE I

|             | Stage I | Stage II | Stage III |
|-------------|---------|----------|-----------|
| Length (cm) | 8       | 20       | 25        |
| Weight (gm) | 30      | 250      | 350       |

TABLE II

*Amino acids of eye lens at different stages of growth of fish Labeo bata*

(Values are expressed in gm/100 gm of dry matter)

| Amino acid     | Stage I | Stage II | Stage III |
|----------------|---------|----------|-----------|
| Alanine        | 3.9     | 3.8      | 3.8       |
| Arginine       | 8.5     | 8.6      | 8.7       |
| Aspartic acid  | 2.4     | 2.3      | 2.3       |
| Cystine        | 1.9     | 2.0      | 2.1       |
| Glutamic acid  | 4.1     | 4.1      | 4.0       |
| Glycine        | 3.7     | 3.7      | 3.7       |
| Histidine      | 2.1     | 2.2      | 2.2       |
| Hydroxyproline | 1.2     | 1.3      | 1.3       |
| Isoleucine     | 1.4     | 1.3      | 1.3       |
| Leucine        | 1.6     | 1.6      | 1.7       |
| Lysine         | 2.3     | 2.3      | 2.4       |
| Methionine     | 1.6     | 1.7      | 1.7       |
| Phenylalanine  | 1.6     | 1.5      | 1.5       |
| Proline        | 2.7     | 2.8      | 2.9       |
| Serine         | 3.5     | 3.5      | 3.6       |
| Threonine      | 2.5     | 2.5      | 2.5       |
| Tryptophan     | 1.3     | 1.4      | 1.4       |
| Tyrosine       | 4.1     | 4.2      | 4.2       |
| Valine         | 2.6     | 2.5      | 2.5       |
| Total nitrogen | 14.4    | 14.5     | 14.5      |

Table II clearly indicates that each of the growth stages contains 19 amino acids of lens proteins. Changes in amino acid contents from one to the other stages are not appreciable. Similarly total nitrogen content too does not change significantly from one stage to the other. At all the three stages of growth, arginine is present in maximum quantities of all the amino acids. At the stage I glutamic acid and tyrosine are present in equal quantities. At the stage II aspartic acid and lysine occur in equal amounts. A comparison with bovine eye lens<sup>2</sup> shows that total nitrogen content of the fish eye lens is lower than that of bovine lens. Bovine eye lens<sup>2</sup> contains only 14 amino acids serine, glycine, alanine, proline and hydroxy proline being absent. Bovine eye lens contains aspartic acid in maximum amount, while arginine content of the fish eye lens is maximum of all the amino acids. In the case of bovine lens arginine content is higher than the aspartic acid. Bovine eye lens contains tryptophan and methionine in equal amounts,

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### IDENTIFICATION OF PYRIDOXINE HYDRO-CHLORIDE FROM MULTIVITAMIN PREPARATIONS

VARIOUS methods<sup>1,2</sup> are described in the literature for the identification of pyridoxine from multivitamin preparations, but none of them is specific. While the method of Hochberg and co-workers is only applicable when pyridoxine is in pure form<sup>3</sup>, method described in various pharmacopoeia using 2,6-dichloroquinone chloroimide is rather a negative test, based upon no colour formation in presence of boric acid. Interference of excipients such as antioxidants,  $\alpha$ -tocopherol and certain preservatives added in multivitamin preparations which give blue colouration in presence of boric acid, limits its application. Colour test described by Feigl<sup>4</sup> using 1,2-naphthaquinone-4-sulfonate, though sensitive, is not specific, as this reagent reacts with all compounds containing two removable hydrogen atoms attached to  $-C$  or  $-N$  atom.

In this paper a new reagent for the identification of pyridoxine in multivitamin preparations by thin layer chromatography is described. 2-Thiobarbituric acid reacts with  $\alpha$ , $\beta$ , unsaturated and aromatic aldehydes<sup>5</sup> giving a pink coloured compound. It is observed that primary alcoholic groups at positions 3 and 4 of pyridoxine can be converted to the corresponding aldehydes by oxidation. The aldehyde when further treated with 2-TBA reagent produces pink coloured complex. No other vitamin of the multivitamin preparation such as vitamin A, thiamine, riboflavin, ascorbic acid, pantothenic acid behaves in a similar way. Vitamin A if present in alcoholic form may interfere but the colour appears after 24 hours on TLC whereas that of pyridoxine appears within 5–10 minutes. However, since vitamin A is only fat soluble it will not interfere with vitamin B<sub>6</sub> where aqueous extract is used. Pyridoxine can also be converted to its aldehyde form using Reimer-Tiemann reaction<sup>6</sup>. The aldehyde is further treated with 2-TBA reagent as described in test-(2).

### EXPERIMENTAL

#### Reagents :

I. Methanol, Acetone and Chloroform—BDH AnalaR.

II. Dichromate in sulphuric acid : 3 g potassium dichromate (AnalaR Grade) dissolved in 100 ml of 30% sulphuric acid.

III. 2-TBA reagent for test (1)—Dissolved 2.88 gm 2-thio-barbituric acid in 100 ml aqueous solution of 0.2 M anhydrous sodium carbonate.

IV. 2-TBA Reagent for test (2)—Dissolved 10 gm 2-TBA in 90 ml perchloric acid and added 10 ml glacial acetic acid.

#### METHOD

*Test (1): Identification on TLC.*—An aqueous extract of the multivitamin tablet was spotted on a 250  $\mu$  layer of silica gel G, activated for half an hour at 110° C. Chromatogram was developed using Methanol : Acetone (7.5 : 2.5) as solvent system. The plate, after drying at room temperature, was first sprayed with potassium dichromate in sulphuric acid which oxidises  $-CH_2OH$  group to  $-CHO$  group giving a green spot. After five minutes the plate was sprayed with 2-thio-barbituric acid reagent. Pink spot appeared after heating the plate for five minutes at 110° C ( $R_f = 0.51$ ). The sensitivity is  $\sim 10 \mu$ g.

*Test (2): Detection of Pyridoxine by spot test.*—To 1.00 ml of pyridoxine solution add 1.0 ml methanolic NaOH (20%) and 1.0 ml chloroform. Keep it in boiling water-bath for five minutes. Allow it to cool to room temperature. Add 3.0 ml of acidic 2-TBA reagent and keep in boiling water-bath for 15 minutes. A pink colour develops. This reagent may be applied for the colorimetric determination of vitamin B<sub>6</sub> in multivitamin preparations (absorption maxima 537.0  $m\mu$ ). The sensitivity of this reagent is  $\sim 100 \mu$ g.

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# STUDIES ON SOME PAPILIONACEOUS SEED OILS

WOLFF AND KWOLEK<sup>1</sup> have pointed out the necessity for collecting more information regarding legume lipids before chemotaxonomic relationships are advanced. Though the genus *Crotalaria* is a large one, consisting of about 300 species, only a few species have been investigated so far, for their lipids. We report here the physical and chemical analysis of three species of *Crotalaria*, namely, *C. juncea* Linn., *C. linifolia* Linn., *C. wightania* along with *Trigonella foenum-graecum* Linn. and *Tephrosia purpurea* Linn. (Pers.).

unsaponifiable matter, by the alkali isomerization method of Hilditch, Morton and Riley<sup>2</sup> using Beckmann DU-2 spectrophotometer. The identification of the fatty acids was carried out by subjecting total fatty acids to reverse phase paper and thin-layer chromatography, impregnating the paper and the plate (Silica gel G) with 10% liquid paraffin in benzene. Acetic acid samples 96% and 90% were used as a solvent systems for TLC and paper chromatography respectively. Iodine vapour was used as the visualising agent and authentic fatty acids were taken as reference for the identification of individual fatty acid.

TABLE I  
Characteristics of the oil

|                          | % of oil | Colour          | Sp. gr. | R <sub>f</sub> index at 25° C | Acid value | Sap. value | Ester. value | Iodine value | % unsap matte |
|--------------------------|----------|-----------------|---------|-------------------------------|------------|------------|--------------|--------------|---------------|
| <i>C. juncea</i>         | .. 12.6  | Greenish-yellow | 0.912   | 1.478                         | 4.6        | 181.2      | 176.6        | 122.2        | 12.6          |
| <i>C. linifolia</i>      | .. 6.2   | do.             | 0.931   | 1.432                         | 3.4        | 179.3      | 175.9        | 107.4        | 13.6          |
| <i>C. wightania</i>      | .. 8.6   | Green           | 0.891   | 1.448                         | 3.8        | 176.8      | 173.0        | 117.6        | 10.3          |
| <i>T. foenum-graecum</i> | 10.3     | Greenish-yellow | 0.962   | 1.468                         | 1.3        | 212.1      | 210.8        | 123.7        | 12.8          |
| <i>T. purpurea</i>       | .. 11.3  | do.             | 0.928   | 1.428                         | 2.6        | 172.5      | 169.9        | 101.4        | 9.5           |

TABLE II  
Composition of fatty acids

|                          |    | Unsaturated fatty acids |                  |              | Saturated acid by difference % |
|--------------------------|----|-------------------------|------------------|--------------|--------------------------------|
|                          |    | Linoleic acid %         | Linolenic acid % | Oleic acid % |                                |
| <i>C. juncea</i>         | .. | 46.8                    | 4.6              | 28.3         | 20.3                           |
| <i>C. linifolia</i>      | .. | 44.6                    | 4.4              | 17.8         | 33.0                           |
| <i>C. wightania</i>      | .. | 48.1                    | 3.8              | 20.4         | 27.7                           |
| <i>T. foenum graecum</i> | .. | 44.9                    | 7.9              | 28.6         | 18.6                           |
| <i>T. purpurea</i>       | .. | 30.8                    | 2.4              | 43.6         | 23.2                           |

Oil content was determined by extraction of 500 gm of powdered seeds in a Soxhlet apparatus for 36 hours with pet. ether (40-60°). Iodine value, saponification value, acid value, unsaponifiable matter, ester value, specific gravity and refractive index were determined by I. P. methods<sup>2</sup>. Linolenic acid and linoleic acid contents were determined spectrophotometrically on mixed fatty acids, free from

The percentage of oil in the six *Crotalaria* species investigated by Kapur *et al.*<sup>4</sup> ranges from 2 to 5.3% while in the species investigated it is much higher. These higher values would push the average value of the oil content in *Crotalaria* species as indicated by Earl and Jones<sup>5</sup> from 3.3%. However, this higher value would still be within the range of the tribe Genisteae which is about 12%. A comparison

of values of oleic acid in the species investigated with those reported by Kapur *et al.*<sup>4</sup> shows a close relationship of *C. linifolia* with *C. agitiflora*, *C. wightiana* with *C. sericea* and *C. juncea* with *C. rubiginosa*. However, it may be noted that the percentage of this acid in *C. mucronata* as reported is only 7.5% which does not seem to fit into the general pattern of the major fatty acid component of leguminosae in general and *Crotalaria* in particular.

Wolff and Kwolek<sup>1</sup> and Gupta and Chakrabarti<sup>6</sup> observed that the compositional difference of fatty acids reflect heridity relationship; however, it may be pointed that it also shows a phylogenetic relationship at the levels of genus and species.

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## STUDIES ON OVALBUMIN AS A DILUENT IN VIROLOGICAL AND SEROLOGICAL TESTS

THE use of bovine albumin in buffered saline as a viral diluent and virus stabilizer in the field of arbovirology has been recommended by various workers<sup>1,2</sup>. The high cost and non-availability of good quality bovine albumin have compelled us to investigate the possibilities of finding a substitute. In the present studies we have used ovalbumin in virological and serological tests and the results are compared with those obtained with bovine albumin (fraction V from bovine plasma, Armour Pharmaceutical Company Limited, England).

Ovalbumin was prepared from white leghorn eggs obtained from Shreekant Poultry Farm, Narayanagaon, Poona, by sodium sulfate extraction method<sup>3</sup>. The purified albumin solution was dialysed against distilled water to remove excess of sodium sulfate. The total proteins were estimated

by the biuret method<sup>4</sup>. Appropriate quantities of albumin solution were then added to phosphate buffered saline and borate buffered saline so as to make 1.5% ovalbumin in phosphate saline (OAPS), pH 7.2 and 0.8% ovalbumin in borate saline (OABS), pH 9.0 respectively.

*Virological Tests.*—The following arboviruses were employed in the study: TR 1751 strain of Dengue type 2, Kaisodi (VRC No. G 14132), African strain of Chikungunya (CHIK), Indian strain of West Nile (VRC No. G 22886), Japanese B Encephalitis (VRC No. P 20778), and Kyasanur Forest Disease (VRC No. P. 9605). Three virus diluents were tested, viz., OAPS, BAPS (0.75% bovine albumin in phosphate buffered saline, pH 7.2) and 1:1 mixture of OAPS and BAPS. All the diluents contained 1000 units/ml of penicillin and 2 mg/ml of streptomycin.

Approximately 5 dex LD<sub>50</sub><sup>5,6</sup> of virus was incubated in each diluent for one hour at 37°C. Further ten-fold virus dilutions were made in the respective diluents. Three weeks old Swiss mice, maintained at VRC, were inoculated by intracerebral (IC) route. Each ten-fold dilution was inoculated into a group of six mice, inoculum being 0.03 ml for each mouse.

1.5% OAPS gave somewhat lower titres, maximum difference being 2.5 dex for P 20778 and minimum being 0.2 dex for P 9605, when compared to those obtained with standard diluent, BAPS. However, 1:1 mixture of OAPS and BAPS was found to be a satisfactory diluent as LD<sub>50</sub> titres for all the viruses tested were almost similar to those obtained with BAPS. When OAPS was employed in 2% concentration the difference in the titres compared to BAPS was considerably reduced.

*Serological tests.*—Haemagglutination (HA) and haemagglutination inhibition (HI) tests were performed according to the methods of Clark and Casals<sup>7</sup>. The following arbovirus antigens were employed in the study: Indian strain of Chikungunya (VRC No. 634029), Sindbis (AR 339), Dengue-1 (Hawaii, DEN-1), Dengue-2 (VRC No. P 23085), Dengue-3 (VRC No. 633798), P 20778, G 22886 and P 9605.

All the antigens were diluted 1:10 in OABS and kept overnight before testing. Another set of antigens was diluted 1:10 in standard diluent—0.4% bovine albumin in borate saline (BABS), pH 9.0 and was also kept overnight before use.

At the optimum pH values, antigens diluted in both the diluents produced similar HA titres. Comparatively higher HA titres were obtained with OABS at higher pH values for P 20778, G 22886, P 23085, and P 9605 antigens. The slight variations

in HA titres obtained at the optimum pH values in the case of AR 339 and P 23085 antigens were within the range of experimental error and thus were not significant.

Homologous mouse hyper-immune sera and fifteen previously tested survey sera (containing 5 human, 5 monkey, 2 bullock, 1 camel and 2 bird) were employed in HI test against all eight antigens to determine the specificity of haemagglutination test with OABS. The tests were performed at optimum pH values of respective antigens for both the diluents. In addition to this, HI test was also performed using OABS at wider pH ranges where comparatively higher HA titres were obtained, e.g., pH 7.0 for P 20778, G 22886 and P 9605, pH 6.0 for DEN-1, P 23085, and 633798. But at these pH values antigens did not remain stable and their titres dropped down. Comparable results were obtained at optimum pH values for both the diluents, viz., OABS and BABS.

Similar results were obtained with different batches of ovalbumin. Ovalbumin being extremely susceptible to denaturation should be stored at 4-8°C and heating, stirring and frothing should be minimised. The precipitate obtained in our stock solution of OABS and OAPS was filtered through filter paper just before use. This, however, did not affect the results.

Our studies indicate that ovalbumin alone can be used for serological tests but needs supplementation with bovine albumin for virological studies.

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# AN ABNORMAL SPECIMEN OF *THYSSA* *MALABARICUS* (BLOCH) (PISCES: ENGRAULIDAE) WITHOUT PELVIC FINS

While examining the Clupeoid fishes from Madras Coast, the author came across a specimen of *Thyssa malabaricus* (Bloch) without pelvic fins (Fig. 1). This is a rare case in Engraulids and hence is being reported here.

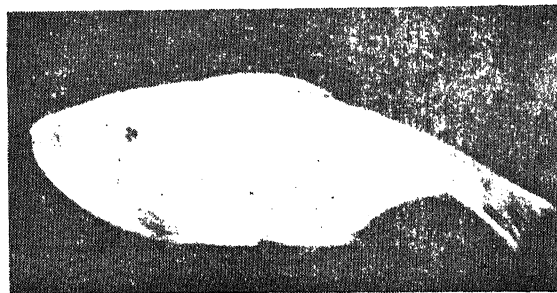


FIG. 1. *Thyssa malabaricus* without pelvic fins.

The meristic counts and the body measurements (in mm) of the specimen are as follows:

P. 13, D. I ii 12, A. ii 38, Scutes (total) 22, G.R. 14 + 17. Total length 139.0, standard length 115.0. Head length 27.3, Body depth 39.2, Eye diameter 7.5, Snout 5.7, Prepectoral distance 29.3, Pectoral fin length 22.5, Preanal distance 70.0, Anal fin base length 37.8, Predorsal distance 55.3, Dorsal fin base length 12.0.

It can be seen that the morphometric characters in general and meristic counts in particular are agreeing with the previous descriptions of this species (Dutt<sup>1</sup>, Whitehead<sup>2</sup>) except for the number of scutes, which is somewhat less in this specimen, perhaps associated with the absence of pelvic fins.

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# STIMULATION IN SEED GERMINATION OF FODDER CROPS THROUGH GROWTH RETARDANTS

THE growth retardants, chlormequat (2-chloroethyl trimethyl-ammonium chloride), B-nine (N-dimethyl-amino succinamic acid), and Phosfon (2, 4-dichlorobenzyl tributyl phosphonium chloride) have been extensively used to study their effect on growth, development and metabolism of plants. In general they produce effects which are reversed from those produced by GA<sup>1-3</sup>. There is no report on the seed germination of fodder crops treated with these substances. Hence, the present study was aimed at finding out the response of these growth retardants on seed germination of fodder crops.

Seeds of cowpea (*Vigna unguiculata* L.) berseem (*Trifolium alexandrinum*), Vetch (*Vicia sativa* L.) and M. P. Chari (*Sorghum bicolor* L.) were separately soaked for 1 hr, 4 hr and 8 hr in 10, 100 and 1000 ppm solutions of these three retardants. For the controls, the seeds were soaked in distilled water for these durations. Seeds were placed between pairs of filter papers put on thick cotton pad in petridishes and left at room temperature ( $25 \pm 5^\circ \text{C}$ ) in the laboratory for germination. Moisture was maintained by adding known quantities of water. Three replications (100 seeds in each replication) were taken in each of the treated combinations. The number of seeds germinated on the 10th day (maximum germination) were

TABLE I  
Growth retardants on seed germination of forage crops

| Treatment                 | hr   | Cowpea | Berseem | Vetch | M. P. Chari |
|---------------------------|------|--------|---------|-------|-------------|
| Control                   | .. 1 | 57.3   | 80.5    | 72.7  | 43.7        |
| do.                       | .. 4 | 59.2   | 83.0    | 80.0  | 48.0        |
| do.                       | .. 8 | 63.3   | 77.0    | 82.8  | 36.5        |
| 10 ppm chlormequat        | .. 1 | 85.0   | 82.0    | 93.8  | 71.0        |
| do.                       | .. 4 | 71.3   | 86.0    | 91.7  | 58.2        |
| do.                       | .. 8 | 49.3   | 78.0    | 53.7  | 39.2        |
| 100 ppm chlormequat       | .. 1 | 93.2   | 90.3    | 95.2  | 84.4        |
| do.                       | .. 4 | 84.2   | 92.6    | 95.0  | 62.0        |
| do.                       | .. 8 | 66.0   | 69.9    | 58.6  | 42.9        |
| 1000 ppm chlormequat      | .. 1 | 85.3   | 74.3    | 89.7  | 45.7        |
| do.                       | .. 4 | 62.6   | 84.7    | 89.3  | 38.7        |
| do.                       | .. 8 | 40.7   | 46.9    | 43.6  | 21.9        |
| 10 ppm B-nine             | .. 1 | 82.6   | 84.3    | 95.2  | 45.0        |
| do.                       | .. 4 | 72.6   | 92.0    | 89.0  | 42.3        |
| do.                       | .. 8 | 51.9   | 50.9    | 50.0  | 31.0        |
| 100 ppm B-nine            | .. 1 | 89.0   | 89.8    | 96.0  | 68.3        |
| do.                       | .. 4 | 81.4   | 95.3    | 95.3  | 48.9        |
| do.                       | .. 8 | 70.7   | 53.4    | 64.0  | 36.3        |
| 1000 ppm B-nine           | .. 1 | 70.0   | 78.0    | 89.0  | 50.0        |
| do.                       | .. 4 | 52.7   | 86.4    | 91.7  | 37.9        |
| do.                       | .. 8 | 32.3   | 42.4    | 49.7  | 24.1        |
| 10 ppm phosfon            | .. 1 | 78.0   | 78.7    | 87.5  | 45.0        |
| do.                       | .. 4 | 74.0   | 89.3    | 62.0  | 34.9        |
| do.                       | .. 8 | 46.0   | 49.7    | 31.0  | 24.0        |
| 100 ppm phosphon          | .. 1 | 70.0   | 83.4    | 83.3  | 38.3        |
| do.                       | .. 4 | 81.4   | 94.4    | 91.0  | 44.6        |
| do.                       | .. 8 | 22.5   | 58.0    | 38.6  | 27.7        |
| 1000 ppm phosfon          | .. 1 | 60.9   | 64.3    | 78.0  | 31.0        |
| do.                       | .. 4 | 41.9   | 71.6    | 63.0  | 33.3        |
| do.                       | .. 8 | 7.2    | 33.5    | 26.3  | 12.7        |
| Concentration C.D. at 5%  |      | 1.50   | 1.44    | 0.78  | 1.41        |
| Soaking period C.D. at 5% |      | 0.79   | 0.76    | 0.99  | 0.79        |
| Interaction C.D. at 5%    |      | 2.60   | 2.49    | 3.08  | 2.46        |

recorded and presented as average per cent seed germination. The critical difference values presented are, however, for the averages of the transformed variables.

The analysis of variance of the transformed data showed that the effects due to the chemicals, concentrations and the soaking durations were significant alongwith the interaction between the chemical concentration and soaking duration (Table I).

Application of the chemicals increased the seed germination percentage in all the crops except for Phosfon application to M. P. Chari. Among the chemicals, Chlormequat and B-nine were better as promoters of seed germination than Phosfon. Generally, the seed germination increased with the increase in the dosage of the chemical from 10 ppm to 100 ppm and was reduced at higher concentration. In cowpea, the increase in the concentration of phosfon, however, resulted in reduced per cent seed germination. In the control, longer soaking duration increased the germination of cowpea and vetch, whereas in berseem and M. P. Chari, the 8 hr soaking resulted in lower per cent germination than even under 1 hr soaking. Increasing the duration of soaking in the chemicals from 1 to 4 hr and 4 to 8 hr resulted in decreased seed germination of cowpea, vetch and M. P. Chari. However, the germination percentage of berseem increased with the increased duration upto 4 hr but in 8 hr soaking, it suddenly decreased and was even lower than that of 1 hr soaking period. In the present study, all the substances employed are surface active substances. They wet the seeds more effectively than plain water and hence better germination in a shorter intervals. The differences in the germination percentage among various seeds may be due to different types of porosity of the membrane covering the seeds. In general, these growth retardants promoted the germination percentage at lower concentrations and inhibited the same at higher concentration (1000 ppm). This stimulation could be attributed to the reduction in the content of inhibitors responsible for delaying or improvement in the permeability of hard seed coat facilitating growth and development of embryos. Plant growth retardants may not act exclusively as growth inhibitors but may also, under certain conditions, stimulate<sup>1-6</sup>. No explanation is offered for these extraordinary results where the effect of a growth retardant shows parallels that of gibberellin.

The results have clearly brought out that the seeds of Cowpea and M. P. Chari soaked in 100 ppm of chlormequat for 1 hr would give the maximum germination of 93.2% and 84.4% respectively. Berseem seeds soaked for 4 hr in 100 ppm of

B-nine gave maximum germination (95.3%) followed by 4 hr. soaking period in 100 ppm phosfon 95.4%. Seeds of vetch also gave maximum germination of 96.0% when soaked for 1 hr in 100 ppm of B-nine.

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#### ROLE OF TANNIN INHIBITORS ON THE INFECTIVITY OF THREE DIFFERENT ISOLATES OF TOBACCO MOSAIC VIRUS

TANNINS are of particular interest and importance in the transmission of plant viruses because of their wide occurrence in different host plants and their virus inhibitory properties<sup>1,2</sup>.

It has been reported that only the purified preparations of the samples from coconut root wilt disease, attributed to a strain of tobacco mosaic virus<sup>3</sup>, is infectious in the form of local lesions on *Chenopodium amaranticolor* Coste and Reyn. while the crude sap is not. Coconut plants are widely known to contain tannin<sup>4,5</sup>. Tannins are known as virus inhibitors<sup>1,2</sup> and possibly playing its inhibitory role as is evident that only the purified preparations from coconut root wilt diseased samples are infectious and not the crude preparations. In view of the above, in the present experiments chemicals which are known to be tannin inhibitors were used to study the effect of these chemicals on the inhibitory influence of the tannin present in coconut host plants, thereby rendering an easy way of isolating the virus from the coconut root wilt affected palms. Numerous methods have been devised from time to time to avoid the inhibitory effects of tannin in transmitting a virus. Infectivity of tannic acid—tobacco mosaic virus (TMV) mixture was restored by adding gelatin<sup>6</sup>. Some alkaloids and soluble and insoluble proteins have also been reported to increase virus infectivity by precipitating tannin<sup>7-11</sup>.



TABLE I

Effect of different concentrations of three chemicals on the infectivity of three isolates of TMV in presence of coconut leaf sap

| Isolate of TMV    | Percentage concentration |      |      |      |           |      |      |      |             |      |      |      |
|-------------------|--------------------------|------|------|------|-----------|------|------|------|-------------|------|------|------|
|                   | Lead acetate             |      |      |      | Polyclear |      |      |      | Hide powder |      |      |      |
|                   | 1                        | 5    | 10   | 15   | 1         | 5    | 10   | 15   | 0.5         | 1    | 2    | 4    |
| Chilli            | 10.4                     | 20.9 | 50.5 | 70.5 | 10.3      | 30.1 | 30.4 | 40.3 | 10.6        | 20.5 | 30.5 | 40.3 |
| Coconut root wilt | 10.1                     | 10.8 | 30.0 | 50.0 | 10.2      | 10.8 | 20.1 | 30.0 | 10.2        | 10.8 | 20.0 | 20.4 |
| Dahlia            | 10.6                     | 20.0 | 20.9 | 60.0 | 10.2      | 10.1 | 20.8 | 30.4 | 10.4        | 30.0 | 40.2 | 60.8 |

Percentage increase in infectivity over control.

Three samples of tobacco leaves infected by three different isolates of TMV, viz., chilli puri orange<sup>1,2</sup>, coconut root wilt<sup>3</sup> and dahlia<sup>1,3</sup> were macerated, the sap extracted and mixed individually with sap obtained from healthy coconut leaves since coconut plants are known to contain tannins<sup>4</sup> (1:1), and supplemented respectively by hide powder, polyclear and lead acetate to ascertain the capacity of these substances to protect the virus from precipitation by coconut leaf tannins. Standard extracts were prepared. All the samples were then centrifuged, the clear supernatants were diluted to 1:100. Four concentrations for the chemicals mentioned above were used. Polyclear and lead acetate showed negligible differences over control at concentrations below 5%; therefore, higher concentrations were tried. On the other hand, hide powder was quite effective even at lower concentrations; therefore more than 5% were not used. Identical treatment was applied to controls, consisting of a mixture of infected tobacco leaves and healthy coconut leaves in 1:1 ratio. *Chenopodium amaranticolor* plants were used for bioassay.

It is evident from Table I that all the chemicals used were found effective and conferred adequate protection on the virus isolates under study from precipitation by the coconut leaf tannins.

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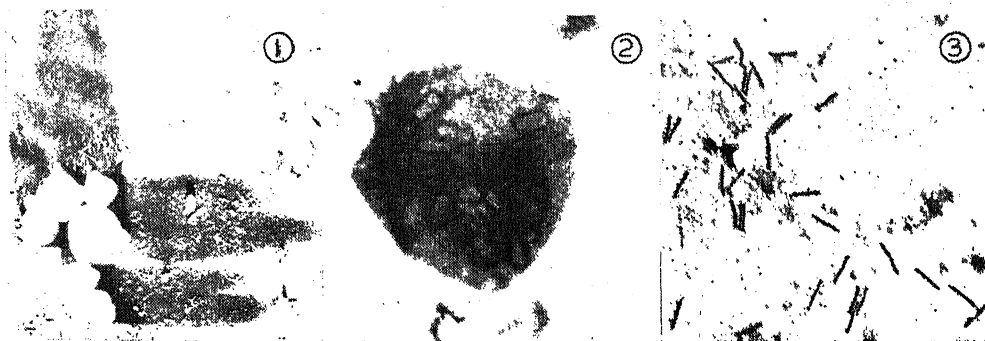
#### NUCLEAR POLYHEDROSIS OF *SPODOPTERA LITURA* (FABRICIUS): DESCRIPTION OF INCLUSION BODIES AND VIRIONS

A NUCLEAR polyhedrosis virus disease was recorded in the larvae of tobacco Caterpillar, *Prodenia litura* F.<sup>1</sup> in India. The species, *eridania*, *litura*, *orthogalii* and *praeifica* belonging to the genus *Prodenia* have been listed to have nuclear polyhedrosis virus disease<sup>2</sup>. In the case of *Prodenia litura*, the first record of nuclear polyhedrosis virus disease is by S. Abul-Nasr<sup>3</sup>. Bergold and Flaschentrager<sup>4</sup> described this virus as *Borrelina litura*. Later Harpaz *et al.*<sup>5</sup> observed that this insect was erroneously referred to as *Prodenia litura* (Fabr.) and referred this species as *Spodoptera littoralis* (Boisduval). C. Boursin<sup>6</sup> while reconstructing the genus *Spodoptera*, renamed *Prodenia litura* as *Spodoptera litura* (F.). The literature on nuclear polyhedrosis virus from 1956–69 contains the reference of *P. litura* though, the species dealt by various workers was *S. littoralis*.

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Hence, it is necessary to publish the virus under investigation in India as a new record of nuclear polyhedrosis virus in *S. litura*.

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Figs. 1-3

The double shadow casted polyhedra (Fig. 1) revealed an irregular form, majority of them appear hexagonal in outline. A few of them were octagonal and tetragonal. The edges of the polyhedra are rounded. The mean diameter of inclusion bodies is  $1.7 \pm 0.29 \mu$  with a range extending from 0.9 to  $2.2 \mu$ .

The partially alkali N.  $\text{Na}_2\text{CO}_3$  dissolved polyhedron stained with 2% phosphotungstic acid (Fig. 2) showed crystallisation pattern which is under investigation. The bundles containing virions are distributed randomly in the protein lattice of the polyhedron. The nuclear polyhedrosis virus of *S. litura* belongs to MNPV group, viz., many nucleocapsids per envelope.

The virions or nucleocapsids appear to be rigid rods. The average length was  $384.8 \pm 41.3 \text{ nm}$  (Fig. 3), and individual virion length ranged from 327.4 to 462.3 nm. The breadth of the virus averaged  $53.6 \pm 5.8 \text{ nm}$  with a range of 42.3 to 66.0 nm.

Philips EM 100 was used for the studies.

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#### PERSISTENCE OF MALATHION RESIDUES IN PADDY PLANTS (*ORYZA SATIVA*)

RESIDUES of malathion on various crops have been determined by many workers. Hopkins *et al.* (1957) on lettuce; Wallis *et al.* (1957) on vegetable berry and tobacco crops; Waites and Van (1958) on turnip, tops, collards, snapbeans and lettuce; Pritam Singh and Rattan Lal (1966) on tomato and pea; Kavadia *et al.* (1968) on Indian rape seed; Hameed and Rattan Lal (1971) on cabbage, cauliflower and knol-khol crops. Anonymous (1960) fixed its tolerance limit 8 ppm on cereals and oilseeds.

For the control of jassids, bugs and other sucking insect pests of paddy, generally malathion in 0.05% concentration at 1000 litres per hectare is used. But information on the deterioration of malathion residues on paddy crop is lacking. Kalode (1969) reported that no measurable residues of malathion could be detected on paddy crop (Taichung Native-1) after 48 hours of application of 0.05% concentration malathion spray.

A study on spray deposits was undertaken by chemical assay method to obtain precise information regarding the persistence of malathion residues on paddy crop, under field conditions.

The experiment was laid out in randomised blocks at Government Usar Reclamation Farm, Chakeri, Kanpur. Each treatment was replicated 4 times. Malathion, in 0.05%, 0.1% and 0.15% concentrations was sprayed @ 1000 litres/hectare on Saket-3 variety of paddy. Spraying was done on the point of slight run off, when grains of the ears were at milk formation stage.

Foliage and ears of paddy plants were plucked one hour, and 1, 2, 3, 5, 7 and 10 days after spraying. Dried leaves and ears of each sample were

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TABLE I

Showing the initial deposits and deterioration of malathion residues in different concentrations in foliage and ears of paddy

| Days after treatment | Residues in ppm     |                      |                      |                      |                      |                      |
|----------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                      | 0.05% conc.         | Percentage reduction | 0.1% conc.           | Percentage reduction | 0.15% conc.          | Percentage reduction |
| 0                    | 37.00               | ..                   | 48.50                | ..                   | 58.50                | ..                   |
| 1                    | 12.00               | 67.58                | 15.00                | 69.07                | 21.50                | 63.25                |
| 2                    | 5.00                | 86.46                | 7.50                 | 84.54                | 13.50                | 76.92                |
| 3                    | Nil                 | ..                   | Nil                  | ..                   | Nil                  | ..                   |
| 5                    | Nil                 | ..                   | Nil                  | ..                   | Nil                  | ..                   |
| Half life (in days)  | 1.3                 |                      | 1.6                  |                      | 2.1                  |                      |
| Regression equation  | $Y = 1.546 - 0.43x$ |                      | $Y = 1.648 - 0.405x$ |                      | $Y = 1.754 - 0.356x$ |                      |

homogenised and measured quantity of finely powdered leaves and ears were used for extraction in Soxhlett extraction apparatus, using carbon tetrachloride as the solvent. The extract was concentrated under reduced pressure and used for the determination of malathion by the technique described by Gunther and Blinn (1955) with the help of Klett Summerson Photoelectric Colorimeter. The log residues (half-life values) of malathion (Fig. 1) was calculated with the formula suggested by Gunther and Blinn (1955).

It is clear from Table I, that as the duration after spraying increases, residues from all the concentrations, viz., 0.05%, 0.1% and 0.15% decreases. The increase in concentrations of malathion spray follows with the increase in residues.

The reduction in the initial deposits of malathion from 0.05% conc. was 12.0 and 5.0 ppm after 1 and 2 days respectively; while in 0.1% conc. it was 15.0 and 7.5 ppm after the same time interval; and for 0.15% conc. it was 21.50 and 13.50 ppm for 1 and 2 days after spraying respectively in all the concentrations. It is significant to note that in all the concentrations on the 1st and 2nd day after spraying, there is about 67-86% reduction on the initial deposits. Malathion residue reaches well below the tolerance limit of 8 ppm within 3 days and completely ineffective within 5 days after spraying on paddy plants.

Half life values of different concentrations of malathion were 1.3 days for 0.05%, 1.6 days for 0.1% and 2.1 days for 0.15%. Dewan *et al.* (1969) have also obtained almost similar results on cowpea fruits and grains.

It reveals that the spraying of malathion in 0.05%, 0.1% and 0.15% concentrations has very little applied interest, as it rapidly decomposes on paddy plants.

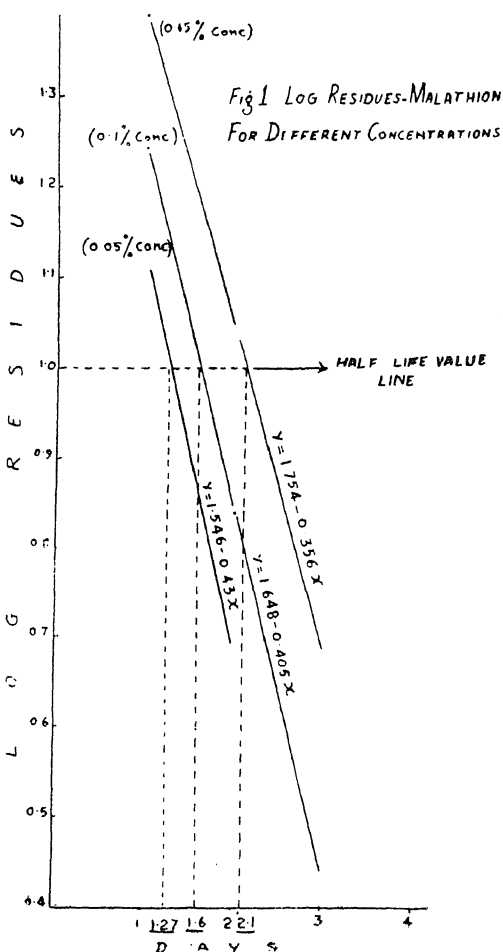


FIG. 1. Log residues Malathion for different concentrations.

Malathion in 0.05%, 0.1% and 0.15% concentrations was sprayed on paddy @ 1000 litres/hectare to study its residual effect. It revealed that the reduction in the toxicity of malathion was 68-87% even on 2nd day of spraying. It reached below 8 ppm within 3 days in all the concentrations. Hence it is not worthwhile to spray malathion on paddy plants.

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### EMS INDUCED MUTATION IN *AMARANTHUS TRICOLOR* L.

MOST of the species of *Amaranthus* L. are cultivated as pot herbs or for their grains. Some of these are polymorphic in nature and certain horticultural varieties have been developed which are of ornamental value. *Amaranthus tricolor* L. is a highly polymorphic one with number of varieties recognised under it. The most variable character in this group of plants is the development of anthocyanin. The pigment develops in various parts of the plants such as stems, petioles, leaves and inflorescences. In the leaves it may develop on the margin or as patches on the lamina giving ornamentations. In some varieties the whole plant is red. As no chromosomal variation was noticed in these forms it was suggested<sup>4</sup> that the development of anthocyanin is under genic control though seasonal variations also affect the development of the pigment within a variety or a species. In a recent attempt to test the mutability in different species of *Amaranthus* L., a fully red coloured variety of *A. tricolor* L. was selected at the first instance.

The seeds were treated with 0.1%, 0.2% and 0.4% of EMS (Ethyl Methane Sulphonate) by soaking for 4 hours in each with a presoaking of 2 hours in distilled water. Then the seeds were thoroughly washed with water and were grown in earthenware pots as well as in petri dishes. A control set was maintained in soaking the seeds in distilled water for 6 hours. Initially the percentage of germination in each concentration was determined and it was found to be 61.0% in 0.1%, 16.0% in 0.2% and 2.2% in 0.4% of EMS as a contrast to 81.1% in control. Thus 0.2% and 0.4% EMS were highly lethal for these seeds. Of the total of 421 seedlings, 5 plants were mutants in M<sub>1</sub> generation which could be distinguished by the complete suppression of anthocyanin development. These seedlings were appearing fully green as a contrast to the complete red colour in normal seedlings. Of these two were from the group treated with 0.1%, 2 from those treated with 0.2% and 1 from those treated with 0.4% EMS but the last one did not survive. However, there were as many as 138 seedlings which showed partial greenness which soon turned red probably due to the recovery of the anthocyanin development. But the complete green seedlings remained green for a pretty long time and though later they developed anthocyanin they could not compete with the normal ones as noticed visually and through spectrophotometric analysis<sup>1</sup>. The anthocyanin was extracted with cold 0.1N HCl for 24 hours. The amount of anthocyanin present in the leaves was estimated by measuring the absorption of the extract at 510 nm (Table I). Thus like chlorophyll mutations<sup>2,3</sup>, anthocyanin development seems to be affected by mutagenic treatment. The four mutants were also marked out from rest of the plants as well as from the control in various other morphological characters (Table II).

TABLE I

Relative anthocyanin content of the leaves of normal and mutant plants

| Sl. No. | Type     | Amount of anthocyanin*    | % of control | % decrease |
|---------|----------|---------------------------|--------------|------------|
| 1.      | Normal   | 0.29<br>(range 0.32-0.26) | 100          | ..         |
| 2.      | Mutant-1 | 0.16                      | 55           | 45         |
| 3.      | Mutant-2 | 0.14                      | 48           | 52         |
| 4.      | Mutant-3 | 0.10                      | 34           | 66         |

\* O.D. at 510 nm per 10 mg fresh weight of leaf.

The cytological analysis of the mutants made through the study of the pollen mother cells also revealed the formation of multivalents in all the mutant plants. While quadrivalents were frequently

TABLE II  
Comparative morphological data of the mutant and normal plants

| Characters                    |    | Mutant   | Normal  |
|-------------------------------|----|--|---|
| Branching                     | .. | Number of equally developed branches from the base | One main shoot with lateral branches less developed |
| Leaf lamina (length/breadth)  | .. | small (3·2/2·2 cm)                                 | Large (7·3/4·5 cm)                                  |
| Flowering (days from soaking) | .. | Early (45 days)                                    | Late (70 days)                                      |
| Seed sterility                | .. | High (69·79 %)                                     | Low (40·19%)  |

noticed in the EMS induced mutant of *Amaranthus dubius* Mart. ex Thell.<sup>2</sup> higher multivalent associations involving 2-7 bivalents have been met with in different cells in these mutants. Such types of meiotic irregularities, however, have been reported<sup>5</sup> to be rare in EMS treatments and more predominant in MES and myleran treatments. The meiotic irregularities might be responsible for the high seed sterility observed in the mutants.

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#### RADIATION INDUCED VARIABILITY IN QUANTITATIVE TRAITS OF JOWAR (*SORGHUM VULGARE*, SNOW.)

MUTAGEN treatment generally induces and increases genetic variability in population. Oka<sup>1</sup> observed significant changes in the means and variability of quantitative traits due to irradiation in rice. Radiation induced variability of several polygenic traits in wheat 'has' also been observed<sup>2</sup>. The increased

genetic variability due to induced mutation may permit selection for desirable traits<sup>3</sup>. With most of the sorghum hybrids having reached grain yield stagnation and others being susceptible to diseases like mildew, leaf blight, leaf spot, and rusts, it is imperative to induce variability in polygenic traits in desirable direction through mutation breeding so as to break the ceiling of yield and improve ideotype in this crop. The present note reports the response of gamma-irradiation in shifting the mean values of several quantitative traits in  $M_1$  generation.

Dry seeds of seven inbreds (CS 3541, 2219 B, IS 3691 B, IS 84, R-16-3, 2077 B and Swarna) and five recently released hybrids (CSH-1, CSH-2, CSH-3, CSH-4 and CSH-5) were treated with gamma-irradiation from  $C_{60}$  source at IARI, New Delhi, with 10 Kr, 20 Kr, and 35 Kr doses. The treated seeds were sown in the field within twenty-four hours of treatment. The observations were made on twenty-five randomly selected plants for each treatment. The height of the plant is reduced due to gamma-irradiation in all the varieties as compared to control, the pronounced reduction in height appeared at 20 Kr and 35 Kr doses. The number of effective tillers per plant was also decreased, the 35 Kr dose reduced the tiller number markedly (30%). There was not much effect of irradiation on the leaf number per plant. The peduncle length in  $M_1$  generation exhibited a decreasing trend in all the varieties excepting Inbred 2219 B and hybrid CSH-2, where no change in peduncle length was noticed. The variety IS 3691 B seemed more sensitive to 20 Kr and 35 Kr doses in comparison to other varieties where flag leaf was just attached to the panicle. All the three doses of gamma irradiation appeared to induce late blooming. The pollen viability and seed fertility data are presented in Table I and these factors decreased significantly in  $M_1$  plants in all the varieties. The 35 Kr dose, however, showed marked effect in hybrid and inbred backgrounds.

TABLE I

*Effects of irradiation on reproductive behaviour of twelve varieties of jowar (Sorghum vulgare Sw.)*

| Treatment             | Varieties |       |       |       |       |        |       |      |         |        |      |        |
|-----------------------|-----------|-------|-------|-------|-------|--------|-------|------|---------|--------|------|--------|
|                       | CSH-1     | CSH-2 | CSH-3 | CSH-4 | CSH-5 | CS3541 | 2077B | 2219 | IS3691B | R-16-3 | IS84 | Swarna |
| Pollen viability (%): |           |       |       |       |       |        |       |      |         |        |      |        |
| Control               | .. 90     | 90    | 77    | 91    | 92    | 77     | 93    | 90   | 86      | 96     | 89   | 76     |
| 10 Kr                 | .. 31     | 78    | 68    | 66    | 87    | 78     | 87    | 35   | 78      | 33     | 88   | 64     |
| 20 Kr                 | .. 77     | 67    | 60    | 53    | 78    | 74     | 72    | 72   | 64      | 77     | 72   | 54     |
| 35 Kr                 | .. 69     | 58    | 43    | 54    | 51    | 68     | 27    | 43   | 56      | 64     | 46   | 40     |
| Seed fertility (%):   |           |       |       |       |       |        |       |      |         |        |      |        |
| Control               | .. 97     | 94    | 82    | 96    | 97    | 82     | 96    | 94   | 91      | 99     | 92   | 80     |
| 10 Kr                 | .. 85     | 82    | 72    | 73    | 92    | 77     | 91    | 89   | 83      | 84     | 91   | 69     |
| 20 Kr                 | .. 79     | 69    | 62    | 59    | 83    | 79     | 75    | 76   | 67      | 87     | 74   | 56     |
| 35 Kr                 | .. 66     | 62    | 47    | 56    | 52    | 71     | 31    | 47   | 60      | 65     | 49   | 42     |

The results suggest a general shift in the mean values in negative direction with regard to the quantitative traits. Some decrease in the biological values of  $M_1$  populations of Sorghum with regard to germination, seedling growth and seed fertility due to the treatment of two chemical mutagens (NMS and MNG) has been observed<sup>5</sup>. A high frequency of diverse types of plant abnormalities were also observed in  $M_1$  generation. More frequent amongst them were chlorophyll streaks, unequal half of leaf blades, thick leaves, and stunted growth. Such  $M_1$  plant abnormalities have also been reported by Shree Ramulu and Goud<sup>7</sup>. There did not seem to be any dependence of mutagenic effects on the backgrounds of the genotypes as the hybrids and inbreds showed similar response to irradiation treatments in  $M_1$  generation in shifting the mean values of quantitative characters. A few dwarf and erect leaved mutants in  $M_2$  generation have been identified. Work on further generations is in progress.

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#### NEW LEAF AND FRUIT GALLS ON *BASSIA LONGIFOLIA* LINN. (SAPOTACEAE)

GALL formation in plants, belonging to the family Sapotaceae in India, appears to be restricted to the genera *Bassia* and *Mimusops* involving two species each, *B. longifolia* Linn., and *B. latifolia* Roxb., *M. elengi* Linn., and *M. hexandra* Roxb. Two midge galls and a chalcid gall are reported from *B. latifolia* while in *B. longifolia* the causative agent in all the four reported galls appear to be midges (Mani<sup>1-3</sup>). Recent observations on gall formation in *B. longifolia* revealed the presence of two more galls—a leaf gall by a midge and a fruit gall by a chalcid, both hitherto unrecorded.

The leaf galls (Fig. 1) appear simple, spherical, pellet-like, pale brown, solid, fleshy, hard, indehiscent, persistent, hypophyllous, 2–3 mm in diameter placed at the ends of the primary lateral veins or of other veinlets, 0.5–1 mm within the margin of the lamina and visible also from the adaxial laminar side. Each leaf bears 2–17 well spaced galls near one or both margins or 2–6 in a closely arranged row. Rarely two adjacent galls may become confluent and appear as a single bilobed structure. The distribution of the galls on either side near the laminar margins is mostly unequal, being of

the order 0-2, 2-5, 0-6, 8-2, 3-8, 17-0, etc., and very rarely subequal.

The bulk of the gall appears to be filled with parenchyma bearing numerous tanniniferous cells, with the nutritive zone surrounding the central circular larval chamber devoid of them. Between the parenchymatous and the nutritive zones is a narrow, 2-3 cell thick, concentric zone of small parenchyma cells, each with a crystal. The vascular strand shows divergently scattered xylem elements terminating at the base of the gall.

Young galled fruits of *B. longifolia* (Fig. 2), appearing about late May, were seen to drop prematurely due to galling by an unidentified chalcid. The weakly lobed, globose fruit galls bear a dense covering of rusty brown hairs. Numerous exit holes—their number depending on the number of emerging adult chalcids—mostly circular, about 1 mm or a little less in diameter, occur around the swollen base of the style (Fig. 2) and also around the base and rarely on the sides of the galled fruit.



FIGS. 1-2. Fig. 1. Pellet-like galls near the leaf margins of *Bassia longifolia* (also seen are the midrib and laminar galls). Fig. 2. Normal and galled fruits (of the same age) of *Bassia longifolia*. Left—normal fruit; middle—galled fruit with dense covering of hairs; right—top view of the galled fruit showing the exit holes.

About 9-40, long often curved or oblique larval chambers of varying sizes with one adult chalcid

or its larva in each, occur inside the galled fruit. In cross-sections, the larval chambers appear circular surrounded by a 6-8 cell thick sclerotic zone and scattered irregularly in the parenchymatous, broadened septa of the galled fruit. Six to nine ovary chambers, usually large and radially arranged, are represented in the galled fruit by as many narrow radiating gaps, lined with one regular row of tanniniferous cells and extending upto half the distance from the centre of the galled fruit.

In heavily infested galls, seed formation is totally suppressed; but in the case of mild infestation (mostly confined to the top of the fruit) one normal seed may develop. It is significant to note that the normal fruits are 1-2 seeded or sometimes 3-4 seeded, indicating that the development of other seeds is being suppressed at an early stage of fruit formation.

Further work on the anatomical aspects of the galls is in progress.

I express my deep sense of gratitude to Prof. T. N. Ananthakrishnan, Loyola College, Madras, for the critical reading of the manuscript and the encouragement and also to Prof. M. S. Mani, St. John's College, Agra, for confirming these galls as new and for identifying the gall makers.

Department of Botany, T. A. LOURDUSAMY,  
Loyola College,  
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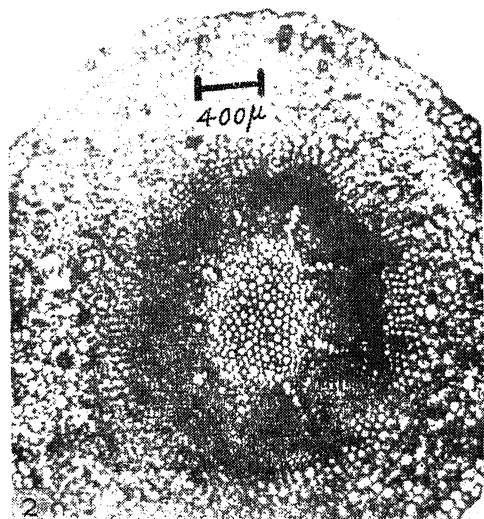
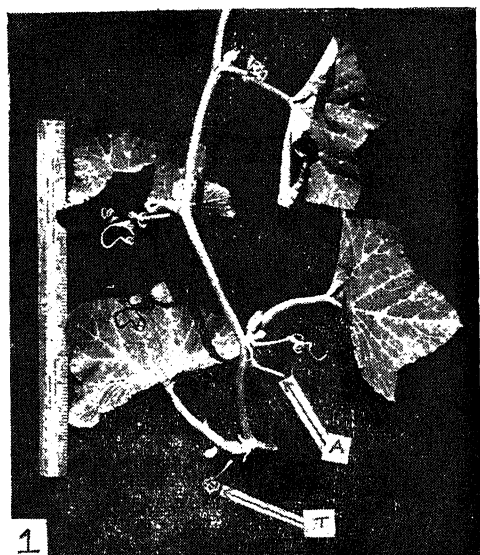
#### AERIAL ROOTS IN *CUCURBITA* L.

HITHERTO, aerial roots in Cucurbitaceae have been reported only in *Momordica*<sup>1</sup>, *Luffa*<sup>2</sup> and *Siolmatra*<sup>3,4</sup>. This note reports the occurrence and describes the anatomy of aerial roots in *Cucurbita maxima* Duchesne.

The plant produces aerial roots at the nodes, below the tendrils. The whitish roots which arise on the upper nodes grow to a length of about 15.0-20.0 cm and measure about 1.5-2.0 mm across. Usually, only one root per node arises, on the upper nodes. They dry out gradually and wither away, apparently being functionless. More than one root per node arise from the lower nodes. They produce lateral roots and develop an extensive system when they reach the ground.

The aerial root is endogenous, arising from the interfascicular region of the stem (Fig. 1). Its epidermis is one cell thick (Fig. 2). The epidermal

cells of the roots, which penetrate the soil, produce root hairs. The cortex is parenchymatous with intercellular spaces. The endodermis and pericycle are each one cell thick. The stele is radial and has four to eight exarch protoxylem strands, alternating with as many patches of phloem. In t.s. (Fig. 2) the phloem patches are large and semicircular in outline. The roots exhibit secondary growth, which is similar to the pattern observed in the normal terrestrial roots of this plant. Periderm formation takes place in older roots and the phellogen is of superficial origin.



FIGS. 1-2. Fig. 1. Part of stem showing aerial roots. A—aerial root, T—tendrils. Fig. 2. Part of t.s. of an aerial root from an upper node.

The roots on the upper nodes die away. Experiments are under progress in this department, to find out whether this intrinsic capacity of this vegetable crop plant could be exploited successfully for fast vegetative propagation.

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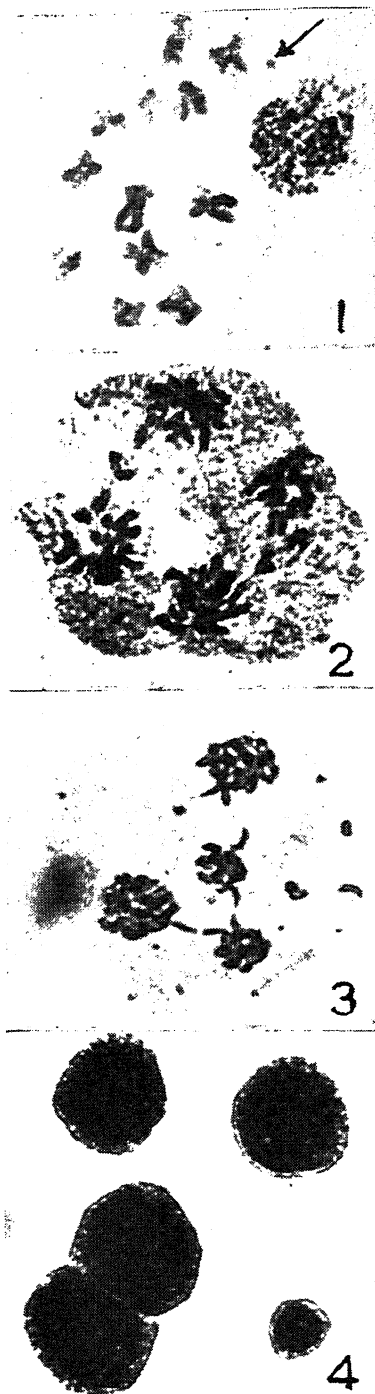
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#### CYTOLOGICAL STUDIES IN *GALPHIMIA GRACILIS* BARTLING

As far as is known, cytological work in the family, Malpighiaceae is restricted to the report of basic chromosome number for *Stigmatophyllon* and *Banisteria*<sup>1</sup>. In view of lack of definite cytological information in the family, it was considered desirable to make a more detailed cytological study of some of the locally available Malpighiaceae. The present communication deals with meiosis in *Galphimia gracilis* (*Thryallis gracilis*) Bartling., a handsome ornamental shrub bearing yellow flowered panicles. In this study, the material collected from the Government College for Women, Guntur, was fixed in 1:3 acetic-alcohol for 24 hours and smeared in acetocarmine.

From the squashes the haploid chromosome number for this species is found to be 12 ( $n=12$ ). Of the 160 cells analysed at diakinesis 79.9% of them manifested 12 bivalents and 10.1% showed a comparatively smaller extra chromosome which appeared to be a B-chromosome (Fig. 1); this being the first report for the family. In about 10% of these cells univalents varying from two to four in number were observed. The bivalents possessed one or two chiasmata and the frequency per cell ranges from seventeen to nineteen. Terminalization coefficient (TC) at diakinesis is 0.33. This low T.C. may be attributed to either terminal non-homology or some other cause. Normal segregation of 12:12 at anaphase I was observed in 65% of microsporocytes. Frequently cells with late disjunction, bridges, bridgefragment configuration (Fig. 3) and lagging univalents (Fig. 2) were also noticed both at anaphase I and II and telophase I and II. Formation of more than four spores (polyspory) was also recorded in about 10% of the dividing microsporocytes (Fig. 4). Possibly this





FIGS. 1-4. Fig. 1. Diakinesis showing 12 II and one B-chromosome,  $\times 1528$ . (B-chromosome arrowed). Fig. 2. Anaphase II with laggards and chromatin bridge,  $\times 1470$ . Fig. 3. Telophase II showing laggards and disjunction bridge,  $\times 1469$ . Fig. 4. Microsporocyte with 5 microspores,  $\times 1785$ .

may be due to the occurrence of lagging chromosomes which may subsequently organise into a micronucleus at telophase I and II. Another feature of interest is the occurrence of dimorphic pollen grains. Of the 600 pollen grains analysed for this feature, 42.6% were comparatively bigger measuring 20.0 to 26.0 microns while 14% were smaller ranging in size from 12.0 to 16.0 microns. In both the cases the pollen grains at anthesis were usually 2-celled, although four nucleate condition was not infrequent and this feature may be due to the precocious mitotic division of the microspore nucleus. Pollen sterility to an extent of 43.6% was recorded. The meiotic abnormalities like lagging, unequal distribution of univalents, bridges with fragments and polypory perhaps account for the partial pollen sterility.

The authors thank Professor A. S. Rao for facilities and taking microphotographs. Our thanks are also due to Dr. B. G. S. Rao for providing literature.

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#### ENERGY CONSERVING EFFICIENCY OF WHEAT IN RELATION TO PLANT COMPETITION

ALTHOUGH the data on energy relations of a number of temperate ecosystems have been published<sup>1-4</sup> so far, the information regarding the terrestrial ecosystems in India is very meagre<sup>5-6</sup>. The present study was carried out with wheat (*Triticum aestivum* L. var. K-68). The crop was grown in two cultivated fields of village 'Moglaha', 7 km north of Gorakhpur University Campus. The water and nutrient supplies were kept above optimum. In one of the fields, designated as Pure Stand (PS), the competing weeds were removed at fortnightly interval, while in the other field, designated as Mixed Stand (MS), the competing weeds were allowed to grow with the crop plants. Net dry matter production of the crop was evaluated by harvesting the plants from five quadrats (25  $\times$  25 cm) for aboveground parts and by digging five soil monoliths (25  $\times$  25  $\times$  30 cm) for underground parts at monthly intervals throughout the growing period and by summing up the positive differences

in the plant biomass. The calorific value of the plant material was estimated using a bomb calorimeter. The energy captured by the vegetation was calculated by multiplying the net dry matter production with its calorific equivalents. The energy conserving efficiency was evaluated by expressing the energy capture as percentages of half of the solar radiation during the period<sup>7-8</sup>.

TABLE I

Energy conserving efficiency (%) of wheat plants in pure stand and mixed stand at different stages of growth

| Growth stages | Age of crop in weeks | Solar energy at land surface (K cal/m <sup>2</sup> ) | Energy conserving efficiency(%) |             |
|---------------|----------------------|--|---------------------------------|-------------|
|               |                      |  | Pure stand                      | Mixed stand |
| Seedling ..   | 4                    | 116400   | 0.55                            | 0.46        |
| Vegetative .. | 8                    | 101400   | 4.19                            | 3.17        |
| Earing ..     | 12                   | 106200   | 11.34                           | 7.54        |
| Maturity ..   | 16                   | 131400   | 3.13                            | 2.63        |
| Harvest ..    | 20                   | 144300   | 0.11                            | 0.09        |

The data set in Table I reveal that the energy conserving efficiency of wheat plants in both the stands increased with the advancing age. But after attaining a certain maximum value it decreased. The maximum value at the earing stage may be due to the maximum net dry matter production at the same stage. This is in conformity with the findings of Dwivedi<sup>5</sup> and Gupta<sup>6</sup>, who have observed maximum energy conserving efficiency at the time of maximum net dry matter production in the case of *Triticum aestivum*, *Dichanthium annulatum* and *Cynodon dactylon* respectively.

The statistical analyses exhibit that the energy conserving efficiency of wheat plants was significantly lower in MS than that in PS ( $t = 1.541$ ;  $P = 0.2$ ). The possible cause for such a reduction may be due to greater competition for light, water and nutrients by weeds in MS. This finding supports the work of Sahai and Das<sup>9</sup>. Similarly, Dwivedi<sup>5</sup> has also observed less energy conserving efficiency in wheat plants of MS than that of PS.

Grateful acknowledgement is made to Prof. K. S. Bhargava for providing facilities and to Rev. Dr. Frank B. Rehnstrom of Sweden for his financial assistance to one of us (L. K. D.).

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#### FATAL EFFECT OF INSECTICIDE-TREATED APHIDS ON COCCINELLA SEPTEMPUNCTATA L.

CONSIDERABLE loss of population of *Coccinella septempunctata* L., an important predator of aphids, may occur when an insecticide is applied to control aphids on crops. The predator suffers in two ways, (i) by coming in direct contact with the insecticide, and (ii) by feeding on aphids poisoned with insecticides. The mortality of the predator by the former factor has been determined by several workers but no work on the latter has been reported so far. The present investigation was, therefore, undertaken to determine the mortality of adult *C. septempunctata* by feeding them on aphids treated with different insecticides.

Fresh mustard leaves heavily infested with aphids were sprayed with one ml of several insecticides in separate petri dishes under a potter's spraying tower at 24 mm mercury pressure. They were allowed to dry for five minutes under a ceiling fan. Sufficient number of aphids were removed with a brush and transferred to unsprayed leaves kept in clean petri dishes. Ten adult beetles from a laboratory culture were released in each petri dish at 20°-25° C. The treatment were replicated five times. Old leaves in each petri dish were replaced by new ones after every 24 hours. The mortality counts of the predator were recorded after 12, 24, 48 and 72 hours. The per cent mortality data thus obtained were corrected by using Abbott's (1925) formula and subjected to statistical tests.

It is evident from Table I that endosulfan, chlorfenvinphos and menazon gave the lowest mortality of the adults of *C. septempunctata*, when aphids

TABLE I

*Comparative mortality of C. septempunctata fed on aphids treated with recommended concentrations of insecticides*

| Insecticide<br>concentration % | Per cent mortality of <i>C. septempunctata</i><br>(Hours after treatment) |             |             |             |
|--------------------------------|---|-------------|-------------|-------------|
|                                | 12  | 24          | 48          | 72          |
| Endrin 0.025 ..                | 16.0 (20.5)   | 36.0 (36.1) | 46.0 (42.0) | 48.0 (43.2) |
| Endosulfan 0.05 ..             | 10.0 (14.3)   | 12.0 (15.6) | 20.0 (23.9) | 22.0 (27.6) |
| Ethyl parathion 0.025 ..       | 38.0 (37.8)   | 42.0 (40.1) | 62.0 (52.6) | 64.0 (53.8) |
| Diazinon 0.025 ..              | 58.0 (49.9)   | 86.0 (73.1) | 96.0 (82.6) | 96.0 (82.6) |
| Menazon 0.05 ..                | 18.0 (22.3)   | 24.0 (29.2) | 32.0 (33.1) | 36.0 (36.5) |
| Malathion 0.06 ..              | 66.0 (54.9)   | 82.0 (67.8) | 90.0 (78.4) | 90.0 (78.4) |
| Chlorfenvinphos 0.05 ..        | 12.0 (20.0)   | 16.0 (23.0) | 24.0 (29.2) | 24.0 (29.2) |
| Formothion 0.03 ..             | 20.0 (25.9)   | 30.0 (32.9) | 48.0 (43.6) | 50.0 (44.8) |
| Dimethoate 0.03 ..             | 12.0 (17.7)   | 40.0 (39.1) | 46.0 (42.6) | 50.0 (45.0) |
| Phosphamidon 0.02 ..           | 24.0 (25.8)   | 32.0 (34.1) | 36.0 (36.4) | 44.0 (41.3) |
| Methyl demeton 0.025 ..        | 40.0 (39.1)   | 40.0 (39.1) | 62.0 (52.1) | 82.0 (65.6) |
| C.D. at 5% ..                  | (9.6)   | (15.5)      | (11.8)      | (11.9)      |

The figures given in parenthesis are the means of angular transformation of per cent mortality in the five replications.

sprayed with these insecticides were fed to them, irrespective of testing intervals.

Aphids sprayed with malathion and diazinon resulted in a high mortality of the beetles at all the testing intervals.

Thus endosulfan, chlorfenvinphos and menazon were the least toxic and malathion and diazinon are the most toxic to the adults of *C. septempunctata*.

The authors are thankful to Prof. D. S. Gupta, for providing necessary facilities and to Shri B. K.

Anand, Division of Entomology, IARI, for identifying the insects.

Haryana Agricultural  
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## SHORT SCIENTIFIC NOTES

### A Note on Prehistoric Cultural Evidence from Tirupati, Andhra Pradesh

This study presents new evidence of prehistoric importance of Tirupati, Chittoor District, based on the author's explorations from early 1973. The western outskirts of Tirupati starting from Sri Venkateswara University campus towards north up to Seven Hills have prehistoric sites with hand axes, cleavers, flakes and a few cores.

Site I is at the rear end of S.V. University Library. Site II is a canal bed on the northern side of S.V.U. Library and the first site. It extends for 12 furlongs in an East-West direction. Here the tools were found at the depth of 4 to 6 feet. Site III is inbetween the second site and the foot of the Seven Hills where a water tank is under construction. All three sites are located within a range of one mile.

It is suggested that this is an evidence of Lower and to a certain extent middle paleolithic cultures in this area. The workmanship and technique of the latter is thought to be 'levellois'. The variety of tools in the three different sites denotes gradual change with improved technology from sites I to III. All the tools are made of Quartzite stone. Its colour varies from reddish brown to grey. The second and third site tools are fresh and original sharpness is retained. Hand axes include different types such as pyriform, conical, cordate, ovate and the like as shown in Table I.

TABLE I

| Category of tools | Site I | Site II | Site III | Remarks                               |
|-------------------|--------|---------|----------|---------------------------------------|
| Hand axes         | 5      | 3       | 4        | Pyriform, Conical, Cordate, and Ovate |
| Cleavers          | 2      | 1       | 3        | Chisel and Rectangular                |
| Flakes            | nil    | 4       | 6        | End and side flakes                   |
| Cores             | nil    | nil     | 3        | ..                                    |

These are dated back to 50 to 75 thousand years based on preliminary analysis.

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### *Spegazzinia sundara* from *Datura metel*

During a regular survey of leaf spot diseases of common medicinal plants, the author obtained a species of *Spegazzinia* which closely resembles

*S. sundara* Subram. Earlier this fungus was recorded on dead bamboos and dead leaves of *Ananas comosus*<sup>1</sup>. Another species, i.e., *S. ornata*<sup>2</sup> also has been recorded on dead leaves.

The present species of *S. sundara*, however, is peculiar in being associated with leaving leaves of *D. metel* Linn. The fungus makes very luxuriant growth on defoliated leaves. Reports regarding the fungus are however very limited. Available literature shows that the fungus was not obtained earlier from *D. metel*.

The author expresses his grateful thanks to Dr. K. S. Bilgrami for laboratory facilities.

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### Induced Polyploidy in Indian Spinach

Indian spinach (*Basella alba*) belonging to the family Basellaceae is a glabrous, succulent herb, mainly used as a green vegetable<sup>1</sup>. Literature on the improvement of this crop to isolate and establish polyploids with enhanced foliage is scanty. Hence an attempt was made to induce polyploidy in this crop and results are summarised in this note.

Growing tips of five day old seedlings of *Basella alba* were treated with 0.25% aqueous colchicine for 72 hours. Out of ten seedlings treated, one plant exhibited distinct gigas characters, characteristic of polyploids and the cytological studies confirmed its autotetraploid nature. Observations on length and breadth of leaves, frequency and size of stomata, size and sterility of pollen grains were gathered from this plant and compared with the normal diploid. The data are presented in Table I.

TABLE I

| Type of plant | L/B ratio of leaves | No. of stomata/unit area | Size of stomata  |                   | Diameter of pollen grains ( $\mu$ ) | Pollen sterility (%) |
|---------------|---------------------|--------------------------|------------------|-------------------|-------------------------------------|----------------------|
|               |                     |                          | Length ( $\mu$ ) | Breadth ( $\mu$ ) |                                     |                      |
| Diploid       | 1.21                | 17.16                    | 3.83             | 2.23              | 2.70                                | 5.00                 |
| Tetraploid    | 1.10                | 12.86                    | 4.03             | 2.25              | 3.20                                | 11.69                |

In general, the autotetraploid was characterised by larger, thicker and succulent leaves, larger stomata and pollen grains. But the number of stomata per unit area and mean pollen fertility were lower than the normal diploid.

Meiosis was studied in the polyploid plant by fixing flower buds of appropriate size in 1:3 acetic alcohol. Chromosome counts made from anther smear preparations, stained with 2% acetocarmine, revealed the autotetraploid nature of the plant, with  $2n = 96$  chromosomes as against  $2n = 48$  in the normal diploid. A total of 41 cells were scored at Metaphase I and Anaphase I, to study the chromosome behaviour. The frequency of quadrivalents and trivalents was much less compared to bivalents and univalents. The mean chromosomal configurations per cell were: quadrivalents 0.31, trivalents 0.052, bivalents 34.84 and univalents 25.21. In spite of large number of univalents observed during Metaphase-I, fairly high degree (68%) normal disjunction of chromosomes to 48:48 was noticed during Anaphase-I, resulting in high percentage of fertile pollen. However, laggarids were also observed at Anaphase-I.

The autotetraploids of *Busella alba* appears to be interesting in view of its high pollen fertility, more thick and succulent leaves. Besides, it can be propagated vegetatively also. Further studies on breeding behaviour and critical evaluation of autotetraploid are in progress.

We are grateful to Dr. N. P. Patil, Director of Research, U.A.S., Bangalore, for his encouragement.

|                           |                      |
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| Hebbal, Bangalore,        | H. S. HANUMANTHAPPA. |

June 26, 1974.

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## ANNOUNCEMENTS

### Award of Research Degrees

Karnatak University, Dharwar, has awarded the Ph.D. degree in Mathematics to Shri Hegde Venkatramana Subraya, for his thesis entitled "Topics in Global Differential Geometry with special reference to Integral Formulae and their applications"; and to Shri Subhas Sangayya Bhusnoormath, for his thesis entitled "Exceptional Values of Meromorphic Functions"; Ph.D. degree in Chemistry to Shri Gurusiddhaya Shivayya Gadaginamath and Shri Muralidhar Gurachar Purohit, for their thesis entitled

"Synthetic Studies in the Indole Field" and "Studies in the Indole Field." respectively.

The M. S. University of Baroda has awarded the Ph.D. degree in Geology to Shri Ramachandra Anant Chansarkar, for his thesis entitled "Drainage and Slope Analysis of Kosi Basin in Central Kumaon with Special Reference to the Geological Controls"; Ph.D. degree in Chemistry to Shri Dineshchandra Ochhavlal Shah, for his thesis entitled "Synthesis of Phenanthro Indolizidines and Nitrogen Mustards as Anti Cancer Agents";

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Mathematics to Shri J. Hanumanthachari, for his thesis entitled "Some Contributions to the Theory of Arithmetic functions"; Ph.D. degree in Physics to Shri V. Kesava Reddy, for his thesis entitled "Studies in Solid State Physics Third-order elastic moduli of Carbon and Alloy Steels"; Ph.D. degree in Zoology to Shri P. Murali Mohan, for his thesis entitled "Studies on the Physiological and Bio-chemical aspects of aestivation of a selected gastropod, with special reference to nervous system"; Ph.D. degree in Botany to Shri K. Subramanya Sastry, for his thesis entitled "Studies on Mosaic Virus Disease of Brinjal incited by a strain of tobacco ring spot virus".

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Physics to Shri M. K. Parida, for his thesis entitled "Description of High Energy Phenomenon"; Ph.D. degree in Chemistry to Smt. Nivedita Mullick, for her thesis entitled "Reactivity of Co-ordinated Ligands"; Ph.D. degree in Botany to Shri Ch. Narasinga Rao and Shri S. N. Ratho, for their thesis entitled "Physiological studies on Tillering Potential in Rice" and "Cytogenetical studies on African cultivated rice (*Oryza glaberrima* Steud) with special reference to the Exploitation of this germplasm" respectively; Ph.D. degree in Zoology to Shri Bimbadhar Nayak, for his thesis entitled "Studies on the Male Germinal Chromosomes of thirty-one species of moths and butterflies (*Lepidoptera*)".

Tamil Nadu Agricultural University, Coimbatore, has awarded the Ph.D. degree in Agriculture to Shri A. Abdul Kareem, for his thesis entitled "Studies on the Antifeeding effects of two organotin compounds, triphenyltin acetate and triphenyltin hydroxide on *Spodoptera litura* F., *Pericallia ricini* F. and *Spomopteryx subsecivella* Zell (Lepidoptera); to Shri M. Gopalan, for his thesis entitled "Studies on Feeding behaviour of Salivary secretions of ragmus importunitas distant (Hemiptera: Miridae) and its influence of the physiology of Sunnhemp, *Crotolaria juncea* L."; to Shri K. R. Ramaswamy, for his thesis entitled "Studies in the Genus *cenchrus* L.".

## REVIEWS AND NOTICES OF BOOKS

Annual Review of Astronomy and Astrophysics (Vol. 12). Edited by G. R. Burbidge, D. Layzer and J. G. Phillips. (Annual Reviews, Inc., 4139, Camino Way, Palo Alto, California 94306), 1974. Pp. 495. Price U.S.A. \$12.00 ; Foreign \$12.50.

The present volume contains seventeen articles. Of these one is on comets, one on the Moon, and three on the Sun. The remaining ones deal with compact X-ray sources, cosmic ray propagation in the Galaxy (collective effects), coherent molecular radiation, large scale dynamics of the interstellar medium, equation of state at ultra high densities, post-main sequence evolution of single stars, the chemically peculiar stars of the upper main sequence, radio radiation from interstellar molecules, the spectra of supernovae, planetary nebulae, nucleo-cosmo-chronology and the origin of light elements.

El-Baz discusses the lunar surface geology in the light of lunar samples obtained during the various moon probes (manned and unmanned). Some interesting photographs accompany the article. Gilman, in his article on solar rotation, discusses in a lucid manner the importance, nature and theories of solar rotation and enumerates the interesting problems that need to be investigated. The article on the solar x-ray spectrum concentrates on wavelengths less than 25 Å. This spectral range is relevant only for the high temperature coronal plasma, in particular active regions and solar flares. Stein and Leibacher discuss in a systematic manner, the various wave modes in the solar atmosphere. An important feature of this article is the description of wave mode properties in terms of the wave number-frequency diagram.

X-ray astronomy was last reviewed in these volumes in 1967. Since then the most spectacular progress has been made in the area of compact x-ray sources. It has been found that a large fraction of the compact x-ray sources consists of members of binary systems. This implies that studies of x-ray stars can provide us with considerable information about the dynamics of binary systems and the properties of compact objects. Litwak, in his review on coherent molecular radiation, discusses strong masers of OH and H<sub>2</sub>O. These masers are important to our understanding of regions of density and size that are intermediate between those of typical interstellar clouds and those of planets and stars. Kaplan and Pikelner

discuss the development of spiral structure and star formation based on large scale dynamics of the interstellar medium. Observational evidence for the theoretical results is briefly reviewed.

Canuto's paper on the equation of state at ultra high densities is the first of the two review articles. The first part concerns the density range  $10^6 \leq \rho \leq 5 \times 10^{14} \text{ g cm}^{-3}$ . The second article is expected to cover the region  $\rho > 5 \times 10^{14} \text{ g cm}^{-3}$ . In view of the numerous mathematical details, this article would appeal only to those who are actively involved in the study of neutron stars. In recent years the discovery of many molecules in space has acquired great importance for several reasons. A whole new field of interstellar chemistry has been ushered in by these discoveries. Zuckerman and Palmer review the radio radiation from interstellar molecules whose radio lines are intimately related to objects under study by optical and infrared observers.

The article by Oke and Searle concentrates on one important and neglected aspect of supernova research, i.e., the description, classification and interpretation of supernovae spectra. The following article on planetary nebulae by Miller traces the important developments since the last review which appeared in these volumes in 1964. In particular, there is a section on new subjects of infrared and radio studies.

Lastly, Reeves shows that by building up the evolutionary abundance curve (i.e., cosmic abundance versus time) of each isotope of the light elements (between hydrogen and carbon) we may obtain a great deal of information not only on their nucleosynthetic processes but also on the features of cosmic, galactic, stellar and even solar system physics that may have altered their abundances or their physical states.

P. K. RAJU.

### Books Received

*Ordinary Differential Equation—A First Course* (2nd Edition). By Fred Braver, John A. Nohel. (Addison-Wesley Pub. Co., Inc., Reading, Massachusetts 01867, U.S.A.), 1973. Pp. ix + 470.

*The Pteridophyte Flora of the Upper Gangetic Plain*. By N. P. Chowdhury. (Navyug Traders, Deshbandhu Gupta Road, New Delhi-5), 1973. Pp. xvi + 90. Price : Rs. 25.00 ; \$ 6.00 ; £ 2.50.

## INFORMATION TO CONTRIBUTORS

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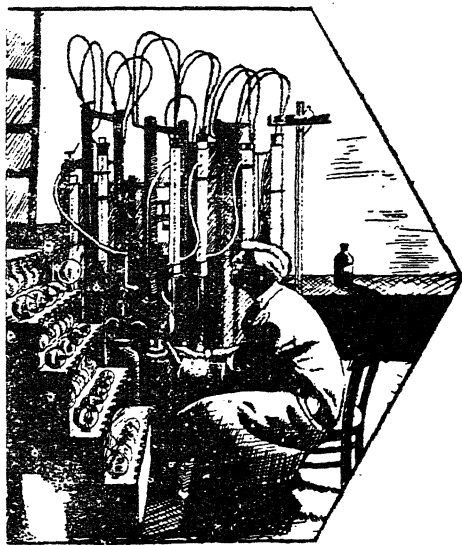
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# STUDIES IN MOLECULAR STRUCTURE SYMMETRY AND CONFORMATION

## V. On the Conformation of the Disulphide Group from X-ray Analysis

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### ABSTRACT

X-ray structural data of compounds containing the disulphide group  $X-S-S-Y$  in open chain molecules are examined with regard to their symmetry and conformation (as well as the chirality in the case of non-centrosymmetric crystals). When  $X$  and  $Y$  are identical and do not have any asymmetric centres in them, the space group in most cases has a centre of inversion or at least a mirror (glide), signifying the presence of both enantiomers. When  $X$  and  $Y$  have both asymmetric centres, the space group is necessarily non-centrosymmetric and enantiomorphous. Eight out of ten cases involving cystine and its derivatives indicate a preference for left chirality while pure cystine in two crystalline forms has right chirality.

### 1. INTRODUCTION

THE conformation of the disulphide group  $X-S-S-Y$  is of considerable interest from several points of view. Its conformation in open chain molecules is well known to be non-planar and dissymmetric similar to that of hydrogen peroxide with the torsion angle  $\theta$  around  $-S-S-$  being around  $\pm 90^\circ$ . In general, when  $X$  and  $Y$  are simple substituents and contain no asymmetric centres such compounds are optically inactive since the two conformers are readily interconverted by rotation around the single bond  $S-S$ , the energy barrier for rotation being small (about 5 kcal/mole). Attempts to resolve the two have therefore not been successful so far. However, if the groups  $X$  and  $Y$  are asymmetric, the two conformers need not have equal energy and consequently one may be preferred over the other. In fact, a typical example is the amino acid L-cystine which shows high negative optical rotation in solution in the visible region, contrasted with moderate positive values shown by most of the other L-amino acids. The high negative rotation of L-cystine has been attributed by Fredga (1950) to the dissymmetric disulphide group.

An earlier analysis by Hordvik (1966) was concerned with correlating the torsion angle  $\theta$  with the bond length  $-S-S-$  and the variation of bond order with the above angle. In the present paper we examine a different aspect of the results of X-ray analysis in the solid state, namely, (a) to study the symmetry and space group of the crystal structures and its dependence if any on the nature of the substituents; (b) When the groups  $X$  and  $Y$  contain asymmetric centres, to study the chirality of the  $-S-S-$  group in the solid state and to compare it with the ORD results in solution. In the latter case the compounds necessarily take up non-centrosymmetric enantiomorphous space groups

and hence the chirality can be established via the X-ray anomalous scattering method (Bijvoet, 1954). The compounds studied in the second category are mostly L-cystine and its derivatives. We exclude from this study compounds which have the  $-S-S-$  group in a ring system or in which  $-S-S-$  acts as a bridge.

### 2. ANALYSIS AND DISCUSSION OF DATA

#### 2.1. Groups $X$ and $Y$ with no Asymmetric Centre

Table I lists the crystal structures and the space group of compounds with  $X$  and  $Y$  containing no asymmetric centres. The first thirteen compounds have  $X=Y$  while the last two have  $X \neq Y$ . It is interesting to note that all but one of these thirteen crystallize in space groups which contain either a centre of inversion or at least a glide plane. This necessarily implies that both conformers exist in these structures. The only exception in the above group is diphenyldisulphide which takes up the enantiomorphous space group  $P2_12_12_1$  so that only one of the two conformers exists in a given crystal. It would be of interest to establish the absolute structures\* of the crystal, so also in the case of the non-centrosymmetric structures such as *p*-bromophenyl disulphide ( $Ccc2$ ).

\* X-ray anomalous scattering effects can be used in general, for any non-centrosymmetric crystal. Thus the effects may be detectable not only in enantiomorphous space groups but also in non-enantiomorphous crystals possessing symmetry element such as a mirror or glide. As has been pointed out elsewhere (Srinivasan, 1971), the term "absolute configuration" in such cases is rather misleading since it connotes, in the conventional sense, one of the non-superposable mirror images, and the term absolute structure would seem preferable. What is established in such cases is the absolute structure in relation to some physical property associated with directional asymmetry.

TABLE I

| No. | Compound  | $\theta$ | formula   | Space group  |
|-----|---|----------|---|--------------|
| 1.  | Diphenyl disulphide [Lee and Bryant (1969)]                                       | 96.2°    | $C_6H_5 \cdot S-S \cdot C_6H_5$   | $P2_12_12_1$ |
| 2.  | <i>p</i> , <i>p'</i> -Dibromophenyl disulphide [Toussant 1945]]                   | ..       | $Br \cdot C_6H_4 \cdot S-S \cdot C_6H_4 \cdot Br$                         | Ccc2         |
| 3.  | <i>p</i> -Dinitrophenyl disulphide [Ricci and Bernal (1969)]                      | ..       | $NO_2 \cdot C_6H_4 \cdot S-S \cdot C_6H_4 \cdot NO_2$                     | C2/c         |
| 4.  | Orthodinitrophenyl disulphide [Ricci and Bernal (1969)]                           | ..       | $NO_2 \cdot C_6H_4 \cdot S-S \cdot C_6H_4 \cdot NO_2$                     | $P2_1/c$     |
| 5.  | 2-2'-Diaminodiphenyl disulphide [Gomes deMesquita (1967)]                         | ± 87°    | $NH_2 \cdot C_6H_4 \cdot S-S \cdot C_6H_4 \cdot NH_2$                     | Pbca         |
| 6.  | Dibenzyl disulphide [Lee and Bryant (1969)]                                       | ± 92.1°  | $C_6H_5 \cdot CH_2 \cdot S-S \cdot CH_2 \cdot C_6H_5$                     | Cc or C2/c   |
| 7.  | 5-5'-Dithiobis (2-nitrobenzoic acid [Sheffer and Kalman (1969)]                   | ± 76.4°  | $NO_2 \cdot (COOH) \cdot C_6H_4 \cdot S-S \cdot C_6H_4 (COOH) \cdot NO_2$ | Pccn         |
| 8.  | Dimethane-sulphonyl disulphide [Sorum 1953]]                                      | ..       | $CH_3 \cdot S(O_2) \cdot S-S \cdot S(O_2) \cdot CH_3$                     | $P2_1/c$     |
| 9.  | Formamidineum disulphide monohydrate (as di-iodide) [Foss <i>et al.</i> , (1958)] | ±104.8°  | $(NH_2)_2C \cdot S-S \cdot C(NH_2)_2$                                     | Pccn         |
| 10. | Formamidineum disulphide (as dibromide) monohydrate [Foss and Johnsen (1957)]     | ±89.2°   | $(NH_2)_2C \cdot S-S \cdot C(NH_2)_2$                                     | $P2_1/c$     |
| 11. | Formamidineum disulphide (as dichloride) [Foss <i>et al.</i> , (1958)]            | ..       | $(NH_2)_2C \cdot S-S \cdot C(NH_2)_2$                                     | Pbca         |
| 12. | Tetraethylthiuram disulphide [Maroy (1965)]                                       | ±88°     | $(CH_3)_2N \cdot C \cdot SS \cdot C \cdot N(CH_3)_2$<br>   S<br>S         | C2/c         |
| 13. | Tetraethylthiuram disulphide [Karle, Estlen and Britts (1967)]                    | ±96.4°   | $(C_2H_5)_2N \cdot C \cdot S-S \cdot C \cdot N(CH_3)_2$<br>   S<br>S      | $P2_1/c$     |
| 14. | <i>t</i> -Butyl N-N-dimethyl trithio percarbamate [Mitchell (1969)]               | ±99.6°   | $(CH_3)_3C \cdot S-S \cdot C \cdot N(CH_3)_2$<br>   S<br>S                | $P2_1/c$     |
| 15. | 2-(2-pyridyl methylidithio) benzoic acid [Karle <i>et al.</i> , 1969]             | 99.1°    | $COOH \cdot C_6H_4 \cdot S-S \cdot CH_2(C_5H_4N)$                         | $P2_12_12_1$ |

TABLE II

| No. | Compound  | $\theta$ | Space Group                                   | Formula  |
|-----|---|----------|---|--|
| 1.  | L-Cystine [Oughton and Harrison (1959)]   | + 73.8   | P6 <sub>1</sub>                               | COOH·CH(NH <sub>2</sub> )·CH <sub>2</sub> ·S—S·CH <sub>2</sub> ·CH(NH <sub>2</sub> )·COOH  |
| 2.  | L-Cystine dihydrobromide [Peterson, Steinrauf and Jensen (1960); Ananthakrishnan and Srinivasan (1964)] | - 81.3   | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> | COOH·CH(NH <sub>2</sub> )·CH <sub>2</sub> ·S—S·CH <sub>2</sub> ·CH(NH <sub>2</sub> )·COOH·2HBr   |
| 3.  | L-Cystine dihydrochloride [Steinrauf, Peterson and Jensen (1958)]                                       | - 79.2   | C2  | COOH·CH(NH <sub>2</sub> )·CH <sub>2</sub> ·S—S·CH <sub>2</sub> ·CH(NH <sub>2</sub> )·COOH·2HCl   |
| 4.  | N-N'-diglycyl-cystine·2H <sub>2</sub> O [Yakel and Hughes (1954)]                                       | - 79.1   | A2  | $\begin{array}{c} \text{O} \\ \parallel \\ \text{NH}_2 \cdot \text{CH}_2 \cdot \text{C} \cdot \text{NH} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{S} - \text{S} \cdot \text{CH}_2 \cdot \\ \quad \quad \quad \mid \\ \quad \quad \quad \text{COOH} \\ \\ \text{O} \\ \parallel \\ \text{CH} \cdot \text{NH} \cdot \text{C} \cdot \text{CH}_2 \cdot \text{NH}_2 \cdot 2\text{H}_2\text{O} \\ \mid \\ \text{OOCH} \end{array}$ |
| 5.  | L-Cystine diamide diHCl [Chaney and Steinrauf (1968)]   | - 81.4   | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> | NH <sub>2</sub> ·CO·CH(NH <sub>2</sub> )·CH <sub>2</sub> ·S—S·CH <sub>2</sub> ·CH(NH <sub>2</sub> )·CO·NH <sub>2</sub> ·2HCl   |
| 6.  | Mono (2, 4-dinitrophenyl) L-cystine [Chaney and Steinrauf (1969)]                                       | ..       | P2 <sub>1</sub>                               | COOH·CH·(NH <sub>2</sub> )·CH <sub>2</sub> ·S—S·C <sub>6</sub> H <sub>4</sub> (NO <sub>2</sub> ) <sub>2</sub>  |
| 7.  | L-Cystine (tetragonal) [Chaney and Steinrauf (1974)]  | + 69.3°  | P4 <sub>1</sub>                               | COOH·CH(NH <sub>2</sub> )·CH <sub>2</sub> ·S—S·CH <sub>2</sub> ·CH(NH <sub>2</sub> )·COOH  |
| 8.  | 4-4'-di (thiouridine) [Shefter and Kalman (1968)]   | - 87.3   | P2 <sub>1</sub>                               | (C <sub>5</sub> O <sub>4</sub> H <sub>9</sub> ) (C <sub>4</sub> N <sub>2</sub> O <sub>1</sub> H <sub>3</sub> )·S—S·(C <sub>4</sub> N <sub>2</sub> O <sub>1</sub> H <sub>3</sub> ) (C <sub>5</sub> O <sub>4</sub> H <sub>9</sub> )  |
| 9.  | di-5 1-(2'-deoxy- $\alpha$ -D-ribo furanosyl) uracilyl disulphide [Shefter, Kotick and Bardos (1967)]   | - 49.1   | P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> | (H <sub>11</sub> O <sub>5</sub> N <sub>2</sub> C <sub>9</sub> ) S—S (C <sub>9</sub> N <sub>2</sub> O <sub>5</sub> H <sub>11</sub> )  |
| 10. | L-cystine dimethyl ester di-hydrochloride monohydrate [Vijayalakshmi and Srinivasan (1975)]             | - 84.4   | P2 <sub>1</sub>                               | $\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3 \cdot \text{O} \cdot \text{C} \cdot \text{CH} (\text{NH}_2) \cdot \text{CH}_2 \cdot \text{S} - \text{S} \cdot \text{CH}_2 \cdot \text{CH} \\ \quad \quad \quad \mid \\ \quad \quad \quad \text{O} \\ \quad \quad \quad \parallel \\ \quad \quad \quad (\text{NH}_2) \text{C} \cdot \text{O} \cdot \text{CH}_3 \cdot 2\text{HCl} \cdot \text{H}_2\text{O} \end{array}$     |

It is of interest to remark here that there was controversial discussion as to whether the space group of dibenzyl-lisulphide is Cc or C2/c [Dijk and Visser (1971); Einsphar and Donohue (1971) and Lee (1971)]. The use of anomalous scattering has resolved this problem (Srinivasan and Vijayalakshmi, 1972).

The spontaneous resolution observed in the case of diphenyl disulphide does not appear to be unique. Similar occurrences have been noted for example in inorganic disulphides (Foss, 1954) and also in organic peroxy compounds (Jeffrey *et al.*, 1964). In fact, the simplest of such a dissymmetric molecule is hydrogen peroxide (Srinivasan, 1970). It crystal-

lises in the enantiomorphous space group P4<sub>1</sub>2<sub>1</sub>2 or P4<sub>3</sub>2<sub>1</sub>2 (Abraham, Collin and Lipscomb, 1951)<sup>1</sup>.

While the enantiomorphous nature of the space group demands in all these cases, only one of the conformers to be present in a given crystal, the possibility of a single crystal containing microdomains of the enantiomorphous regions is not ruled out at least in a few cases where conditions are favourable. In fact, Sax and McMullan (1967) suggested the possible existence of such microdomains in the case of dibenzoyl peroxide considering the diffuse scattering intensity and the geometry of packing of the molecule in the unit cell. The above possibility may be verifiable by the use

of X-ray anomalous scattering technique, since the presence of equal amounts of each domain should give practically zero Bijvoet differences. This aspect is being pursued in this laboratory.

The last two compounds have  $X=Y$  and one of them takes up the enantiomorphous space group  $P2_12_12_1$ , while the other is centrosymmetric ( $P2_1$ ).

## 2.2. Group A, Y with Asymmetric Centres

Table II lists compounds with X and Y containing asymmetric centres. In all the cases excepting (6), the two groups are the same ( $X=Y$ ). As is to be expected they all take up non-centrosymmetric enantiomorphous space groups. Eight of the cases are L-cystine derivatives. Since the absolute configuration of the L-amino acid is known from X-ray anomalous dispersion method, this may be used as a reference to deduce the absolute conformation of the disulphide group in these crystals. In five of these cases, namely, (2) to (5) and (10), the chirality of the disulphide group is left (dihedral angles negative, around  $-80^\circ$ ) while in the two forms (1) and (7) of pure L-cystine alone it is right (positive, namely  $71^\circ$ ). Structural details for (6) are lacking. The two compounds (8) and (9) which are not cystine derivatives also have left chirality.

Thus, while all the derivatives of L-cystine indicate a preference for left chirality, for pure cystine alone (both hexagonal and tetragonal forms) the indication is to the contrary. A firm conclusion is therefore not possible although, if we ignore differences between the pure form and the derivatives, there is an overall preference for left chirality.

These may be taken to be in broad agreement with the results of ORD and CD studies in solution for disulphide group in ring system (Carmack and Neubert, 1967; Claeson, 1968; see also Linderberg and Michl, 1970). However these have to be reckoned with caution since our data concern primarily with open chain molecules. Semi-empirical methods for energy calculations may be expected to throw light on this aspect. These are being investigated and the results will be reported in due course.

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# ULTRASONIC STUDY OF ASSOCIATION IN DICARBOXYLIC ACIDS

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## ABSTRACT

Using Jacobson's free length theory, association factors in dicarboxylic acids have been calculated. For this purpose ultrasonic velocities and temperature coefficients of velocity of six acids in the homologous series are measured, using the composite ultrasonic interferometer designed in this laboratory. It is found that these acids obey Lagemann's rule, when association is taken into account.

THE ultrasonic behaviour of monocarboxylic acids has been reported earlier in a series of communications<sup>1-3</sup> from this laboratory and these studies are now extended to the homologous series of dicarboxylic acids with a view to studying the similarities and differences, if any, between these two groups of substances. Interesting results are obtained and several peculiar differences exist between monocarboxylic and dicarboxylic acids in their ultrasonic behaviour. Some of the important results are reported in this communication.

The temperature variation of ultrasonic velocity and density of these acids are shown in Figs. 1 and 2 respectively and their temperature coefficients are given in column 4 of Table I. The density of the dicarboxylic acids is found to decrease with increase in molecular weight, a behaviour similar to that of normal fatty acids<sup>3</sup>. The ultrasonic velocity decreases and temperature coefficient of velocity increases with increasing molecular weight in dicarboxylic acids, while the opposite is the behaviour in monocarboxylic acids<sup>1</sup> where velocity

TABLE I

| Acid          | Molecular weight | Melting point* (°C) | $-\frac{\Delta c}{\Delta t}$ [m/sec/°C] | $C_{170^\circ\text{C}}$ (m/sec) | $\rho_{170^\circ\text{C}}$ (gm/cc) |
|---------------|------------------|---------------------|---|---------------------------------|------------------------------------|
| Glutaric Acid | 132.11           | 97.5                | 2.402                                   | 1169                            | 1.150                              |
| Adipic Acid   | 146.14           | 151.0               | 2.442                                   | 1147                            | 1.088                              |
| Pimelic Acid  | 160.17           | 103                 | 2.472                                   | 1121                            | 1.049                              |
| Suberic Acid  | 174.19           | 140                 | 2.491                                   | 1102                            | 1.014                              |
| Azelaic Acid  | 188.22           | 106.5               | 2.506                                   | 1090                            | 0.983                              |
| Sebacic Acid  | 202.25           | 133                 | 2.517                                   | 1082                            | 0.961                              |

\* Values are taken from *Hand Book of Chemistry and Physics* (1959) (Chemical Rubber Publishing Company, Cleveland, Ohio).

A composite ultrasonic interferometer<sup>4</sup> is used for determining ultrasonic velocities and temperature coefficients of velocity of six dicarboxylic acids, namely, glutaric, adipic, pimelic, suberic, azelaic and sebacic acids. As all the acids have high melting points (as shown in column 3 of Table I) the ultrasonic cell is kept in a special oil bath, whose temperature is regulated within  $\pm 0.3^\circ\text{C}$  by a mercury regulator, designed in this laboratory. The whole experimental set-up is well shielded to attain thermal equilibrium. As all the substances are either Reidell or B.D.H. samples of 'Analar' purity, no further purification is attempted. The velocities and their temperature coefficients are accurate to 1 in 1000. The densities are determined correct to the third decimal place, employing a specially designed dilatometer, which records changes in volume with temperature of a known mass of the substance.

increases and temperature coefficient of velocity decreases. The magnitude of  $(\Delta c/\Delta t)$  is smaller than that of monocarboxylic group of acids<sup>1</sup>.

Using the free length theory of Jacobson<sup>5</sup>, association factors of these acids have been calculated at  $170^\circ\text{C}$ , at which temperature, all of them are in the liquid state. According to Jacobson, the intermolecular free length ( $L_f'$ ) in liquids is related to the adiabatic compressibility ( $\beta_{ad}$ ) by the relation

$$L_f'^2 = K^2 \cdot \beta_{ad} \quad (1)$$

where K is a temperature dependent constant. The values of K at different temperatures upto  $50^\circ\text{C}$  have been given by Jacobson<sup>5</sup>. Wada<sup>6</sup> found that K is proportional to  $T^{1/2}$  where T is the temperature in absolute degrees and that  $(K/T^{1/2})$  is equal to a value of 36, with a variation of 1% in the temperature range  $0^\circ\text{C}$  to  $50^\circ\text{C}$ . Subsequently, Swamy<sup>7</sup>

TABLE II

| Acid          | $V_{170}^0$<br>(cc) | $V_f$<br>(cc) | $L_f \times 10^8$<br>(cm) | $\beta_{ad} \times 10^{12}$<br>(at 170°C<br>[cm <sup>2</sup> /dyne]) | $L_f' \times 10^3$<br>(cm) | $\alpha$ | $[xM]^{1/2} \Delta c / \Delta t$<br>[gm <sup>1/2</sup> /m.<br>sec <sup>-1</sup> °C <sup>-1</sup> ] |
|---------------|---------------------|---------------|---------------------------|--|----------------------------|----------|--|
| Glutaric acid | 114.87              | 95.10         | 0.4647                    | 63.65  | 0.6046                     | 2.191    | 40.83  |
| Adipic acid   | 134.28              | 109.6         | 0.5277                    | 69.93  | 0.6339                     | 1.732    | 38.83  |
| Pimelic acid  | 152.72              | 124.1         | 0.5634                    | 75.87  | 0.6603                     | 1.610    | 39.71  |
| Suberic acid  | 171.79              | 138.7         | 0.6049                    | 81.23  | 0.6831                     | 1.439    | 39.42  |
| Azelaic acid  | 191.45              | 153.1         | 0.6561                    | 85.61  | 0.7013                     | 1.221    | 38.00  |
| Sabacic acid  | 210.41              | 167.5         | 0.6910                    | 88.96  | 0.7148                     | 1.109    | 37.69  |

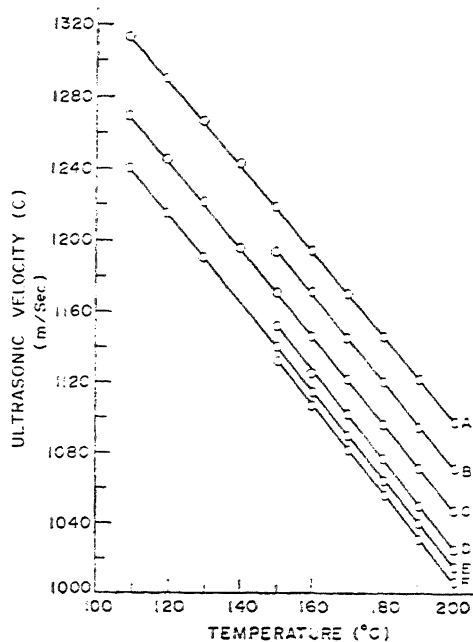


FIG. 1. Temperature variation of ultrasonic velocities. A, Glutaric acid; B, Adipic acid; C, Pimelic acid; D, Suberic acid; E, Azelaic acid; F, Sebacic acid.

extended the validity of Wada's relation to higher temperatures. He found that  $(K T^{\frac{1}{2}}) = 36$  with a maximum variation of 1.5% upto a temperature of 157°C. Assuming the validity of Wada's linear relation between  $K$  and  $\sqrt{T}$  in the present study, the value of  $K$ , corresponding to the temperature of 170°C, is found to be equal to 758.

According to general definition of free length

$$L_f = \frac{2(V_T - V_0)}{[36\pi N V_0^2]^{1/3}} \quad (2)$$

where  $V_T$  is the volume at temperature  $T^\circ K$ ,  $V_0$

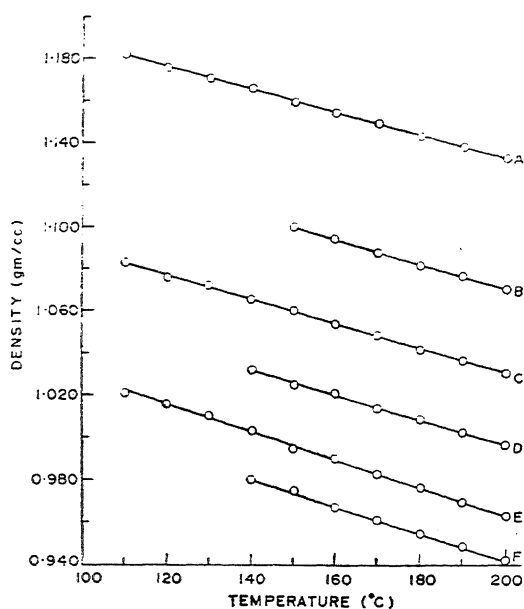


FIG. 2. Temperature variation of densities of dicarboxylic acids.

is the volume at absolute zero and  $N$  is the Avogadro Number.

Volumes  $V_{170}^0$  at 170°C for each liquid is calculated by evaluating its density  $\rho_{170}$  from the density graphs shown in Fig. 2.  $V_0$  is evaluated from Sugden's zero volume contributions. Values of  $V_{170}^0$  and  $L_f$  for different liquids are shown in columns 2.3 and 4 respectively of Table II.

The values of  $\beta_{ad}$  calculated for different liquids at 170°C and the values of  $L_f'$  calculated from equation (1) are shown in columns 5 and 6 respectively of Table II. A plot of  $L_f^2$  against  $\beta_{ad}$  for different liquids with  $K = 758$  is shown in

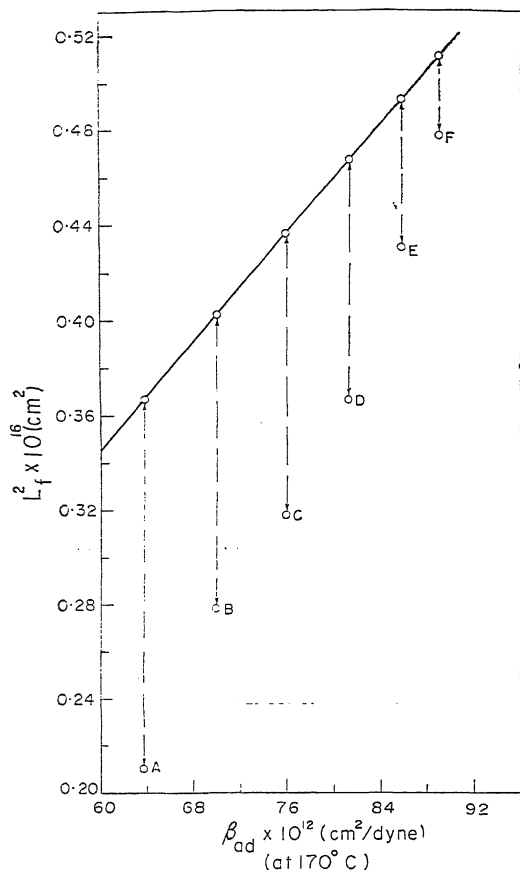


FIG. 3. Relationship between adiabatic compressibility and intermolecular free length of dicarboxylic acids at 170°C.

Fig. 3. All the evaluated points for different substances are found to lie below the straight line.

The association factors  $\alpha$  calculated for different substances by the relation

$$\alpha = \left[ \frac{L_f}{L_f'} \right]^3$$

are shown in column 7 of Table II. It is interesting to observe that association decreases with increase of molecular weight of the acid. When association is taken into account, Lagemann's rule<sup>9</sup> for normal liquids is obeyed by these substances as well and the product  $[\alpha M]^{\frac{1}{3}} \frac{L_f}{L_f'}$  is found to be constant as shown in column 8 of Table II with an average value of 39.08.

The first three members of the homologous series of dicarboxylic acids decompose near their melting points and as such their ultrasonic behaviour is being studied by solution method and their results will be published elsewhere.

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## NUTRIENT COMPOSITION OF BARBADA OR MALMANDI (*INDIGOFEA GLANDULOSA*)

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#### ABSTRACT

The seeds of *Indigofera glandulosa*, a wild legume grown in some parts of Deccan region, have been analysed for nutrient composition and amino acid profile. The legume contains 26% protein and is a good source of minerals and B-complex vitamins. Aminogram and bioassay for acute toxicity reveal that the seeds do not contain undesirable constituents. The possibilities of exploiting such innocuous wild legumes with good nutritive potential for edible purposes have been discussed.

**I**NDIGOFEA GLANDULOSA, commonly known as barbada, malmandi or befri is an annual herb found in parts of U.P., Bihar, Gujarat and the Deccan plateau. It has been described as nutritious and is believed to possess the qualities

of a tonic in Indian medicine<sup>1,2</sup>. Seeds of this wild legume are reported to contain 31% protein and cultivated varieties appear to contain even higher amounts<sup>3</sup> (37%). However, complete information on the nutrient composition and the



essential amino acid profile of this legume is not available. In view of the reported good qualities of this legume, it was of interest to investigate in

## RESULTS

The data on the nutrient composition are indicated in Tables I and II.

TABLE I

Nutrient composition of seeds of *Indigofera glandulosa*, compared with other common pulses

| Nutrient            | <i>Indigofera*<br/>glandulosa</i><br>(malmandi) | Chick pea<br>(Chena) | Pigeon pea<br>(Tuar) | Green gram<br>(Mung) | Black gram<br>(Urad) |
|---------------------|---|----------------------|----------------------|----------------------|----------------------|
| Protein g%          | 26.1  | 17.1                 | 22.3                 | 24.0                 | 24.8                 |
| Total ash g %       | 2.18  | 3.0                  | 3.5                  | 3.5                  | 3.2                  |
| Calcium mg 100 g    | 154   | 202                  | 73                   | 124                  | 154                  |
| Phosphorus mg 100 g | 291   | 312                  | 304                  | 326                  | 385                  |
| Ca : P              | 1:1.9   | 1:1.5                | 1:4.2                | 1:2.6                | 1:2.5                |
| Iron mg 100 g       | 22.4  | 10.0                 | 5.8                  | 7.3                  | 9.3                  |
| Molybdenum mg 100 g | 2.76  | 1.95                 | 2.22                 | ..                   | ..                   |
| Thiamin mg 100 g    | 0.8   | 0.30                 | 0.45                 | 0.47                 | 0.42                 |
| Riboflavin mg 100 g | 0.31  | 0.15                 | 0.19                 | 0.27                 | 0.2                  |
| Niacin mg 100 g     | 4.47  | 2.90                 | 2.90                 | 2.10                 | 2.00                 |

\* Data on the basis of present investigation.

Data on other pulses drawn from *Nutritive Value of Indian Foods*.<sup>9</sup>

detail the nutrient composition, the essential amino acid content and also to examine the possible presence of unusual toxic amino acids.

## MATERIALS AND METHODS

Sample of seeds of *Indigofera glandulosa* was obtained by the courtesy of the Department of Extension of the Ministry of Agriculture, Southern Region, Bangalore.

Proximate principles were determined by standard techniques<sup>4</sup>. Mineral and trace element composition were determined by methods described by Sandel<sup>5</sup>. The B-complex vitamins were estimated by fluorometric or microbiological methods<sup>4</sup>. Essential amino acids were assayed by both microbiological assays and by the use of automatic amino acid analyser, using appropriately processed protein hydrolysates of the sample<sup>6</sup>. Free amino acids were examined by two-dimensional paper chromatographic method using extractives of 70% alcohol, using butanol: acetic acid: water (12: 3: 5) and phenol: water (4: 1) with  $\text{NH}_4\text{OH}$  as described by Ivor Smith<sup>7</sup>.

Acute toxicity studies on extracts from 70% alcohol extract were carried out on day-old chicks by intraperitoneal administration of appropriate dosages of the extract concentrates<sup>8</sup>.

It can be seen from the results that malmandi is a good source of protein. The protein, however, is deficient in methionine and in tryptophan. Like other legumes, it is a good source of lysine. Arginine content in the seed protein is very high, being threefold higher than the levels seen in other legumes. In regard to the mineral composition, it compares favourably with the most of the commonly used pulses. It is a better source of iron and B-complex vitamins. The amino acid profile determined by both the automatic amino acid analyser and the two-dimensional paper chromatography did not reveal the presence of any unusual amino acid.

Acute toxicity studies by daily intraperitoneal administration of concentrates of 70% alcohol extracts to baby chicks for about a week did not indicate any demonstrable signs and symptoms of toxicity.

## DISCUSSION

The seeds of the wild legume, *Indigofera glandulosa*, are a good source of protein, minerals and vitamin B-complex. The quality of protein as measured by the amino acid profile compares favourably with other pulses which are commonly used. Since the crop is drought resistant and can be grown in dry

TABLE II

Essential amino acid composition and protein quality of malmandi compared with other pulses

| Amino acid                | <i>Indigofera</i> *<br><i>glandulosa</i><br>(malmandi) | Chick pea<br>(Chena) | Pigeon pea<br>(Tuar) | Green gram<br>(Mung) | Black gram <sup>‡</sup><br>(Urud) |
|---------------------------|--|----------------------|----------------------|----------------------|-----------------------------------|
| Arginine g/16 g N         | 24.7   | 9.12                 | 5.76                 | 8.0                  | 8.32                              |
| Lysine                    | 7.12   | 7.04                 | 7.68                 | 7.36                 | 6.40                              |
| Threonine                 | 4.92   | 3.52                 | 3.20                 | 3.20                 | 3.52                              |
| Methionine                | 0.84   | 1.28                 | 0.96                 | 1.28                 | 1.44                              |
| Tryp ophan                | 1.03   | 0.80                 | 0.69                 | 0.96                 | 1.12                              |
| Leucine                   | 8.56   | 9.28                 | 7.9                  | 8.11                 | 8.00                              |
| Isoleucine                | 4.69   | 5.12                 | 4.00                 | 5.60                 | 5.44                              |
| Lecuc/Isoleucine          | 1.82   | 1.81                 | 1.80                 | 1.44                 | 1.47                              |
| Histidine                 | 3.91   | 2.56                 | 4.0                  | 2.72                 | 2.72                              |
| Phenylalanine             | 4.17   | 5.76                 | 7.36                 | 5.6                  | 4.96                              |
| Valine                    | 5.1  | 4.96                 | 4.16                 | 5.12                 | 4.96                              |
| Biological value†         | 50.52  | 68                   | 57                   | 54                   | 63                                |
| Protein efficiency ratio‡ | 1.02   | 1.7                  | 1.5                  | 0.8                  | 1.0                               |
| Digestibility§            | 83%  | 82-86%               | 85%                  | 86-94%               | 83-85%                            |

\* Data on the basis of present investigation; † B. V. reported; ‡ P.E.R. reported; § Data reported<sup>10,11</sup>.

regions, it appears to be a good and low-cost source of nutritious food. Since it is a good source of B-complex vitamins, particularly niacin, consumption of this pulse, in combination with jowar, could control endemic pellagra seen among the low income groups.

The arginine content of this seed is unusually high. Several other hardy legumes such as *Lathyrus* and *Canavalia* species which are also drought resistant crops contain appreciable amounts of higher homologues of arginine<sup>12</sup>.

$\beta$ -Nitropropionic acid, a neurotoxic amino acid from *I. enneaphylla*<sup>13</sup> and indospicine, a hepatotoxic amino acid from *I. spicata*<sup>14</sup>, have been reported to restrict the utilisation of these species as food for cattle or human. Long term studies are now under progress to examine any possible untoward effects due to prolonged consumption of the seeds of *Indigofera glandulosa*.

## ACKNOWLEDGEMENTS

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## LETTERS TO THE EDITOR

## HOLOGRAPHIC FORMATION OF EQUAL INCLINATION AND EQUAL THICKNESS FRINGES AND ESTIMATION OF REFRACTIVE INDEX OF SOLUTION

IN principle fringes of equal inclination can be formed if two parallel wavefronts, one lagging behind the other, are made to interfere and equal thickness fringes can be obtained when the interfering wavefronts are inclined to one another. Holographic interferometric method has been employed here to form them. While forming fringes of equal inclination, a cell with solutions of different concentration was placed in the path of the object beam to produce phase difference between the interfering wavefronts and thus an attempt has been made to compare refractive indices of solutions at different concentration.

A schematic diagram of the usual off-axis holographic experimental set-up is shown in Fig. 1. A 15 mw He-Ne laser is used as a source of coherent light. The laser beam is split into two by means of a beam-splitter. After expansion one beam directly goes on to the photographic plate and the other reaches the plate through the liquid cell. Polaroids are introduced in each beam to control their intensity ratio. The functions of the other optical components in the set-up are well known.

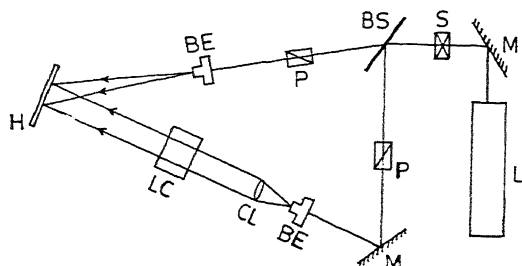


FIG. 1. Schematic diagram of the experimental set-up. L, Laser; M, Mirrors; S, Shutter; BS, Beam splitter; P, Polaroids BE, Beam Expanders; CL, Collimating Lens; LC, Liquid-Cell; H, Photographic Plate.

A double exposure hologram was made by giving the first exposure with the cell containing distilled water and the second exposure after replacing distilled water by a salt solution of known concentration. On reconstruction circular fringes have been observed. A set of similar holograms were made using solution of various concentrations and found that the number of fringes in each case

was proportional to the concentration of the solution. Figure 2 shows a typical system of circular fringes. In the second case the liquid cell is replaced with a plane parallel glass plate with a provision to measure its angle of rotation accurately. The first exposure was given when the plate was normal to the object beam and the second exposure after rotating the plate through a known angle. Reconstruction of the hologram has revealed a set of straight fringes which are shown in Fig. 3.



Fig. 2. Circular fringes for 10 gm of NaCl in 100 cc of distilled water.

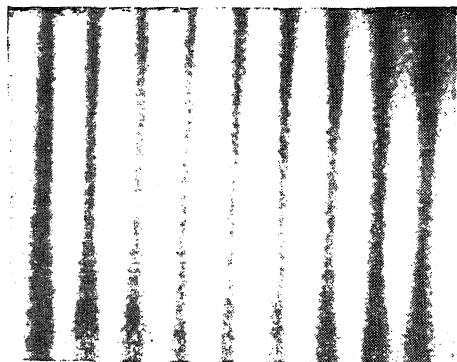


FIG. 3. Straight fringes for 1.0° Tilt.

On the basis of the theory pertaining to the double exposure hologram the intensity distribution in the circular fringe system has been deduced to be

$$I = 2a^2 \left[ 1 + \cos \left( \frac{2\pi d}{\lambda} \cos \theta \right) \right]$$

where  $a$  is the amplitude of the object beam,  $d$  the path difference and  $\theta$  the angle of inclination with the axis. It is evident from this expression that the

fringes obtained in this case should be circular and are of equal inclination type.

Similarly, the intensity distribution in the case of straight fringes may be expressed by

$$I = 4a^2 \cos^2 \frac{\delta}{2}$$

where  $\delta$  is the phase difference. This equation clearly indicates that these fringes can be considered as fringes of equal thickness.

On the basis of a well known relation<sup>1</sup>  $\mu t \cos \theta = n\lambda$  we have established a relation between refractive indices of two solutions at different concentrations by making use of the circular fringes. If  $r$  and  $r'$  are respectively their refractive indices, their ratio is given by

$$\frac{\mu'^2}{\mu^2} = \frac{n_1^2 r_2^2 (r_1'^2 - r_1'^2) - n_2^2 r_1'^2 (r_2'^2 - r_2'^2)}{n_1^2 r_2'^2 (r_1'^2 - r_1'^2) - n_2^2 r_1'^2 (r_2'^2 - r_2'^2)}$$

In this formula  $r_1, r_2$  are the radii of  $n_1$ th ring and  $r_1', r_2'^2$  are the radii of  $n_2$ th ring in each case.

The following are the various values obtained for different variables of the above equation.

(1) For 20 gm of NaCl in 100 cc of distilled water

$$\begin{array}{ll} n_1 = 2 & n_2 = 5 \\ r_1 = 1.3 \text{ cm.} & r_1 = 3.0 \text{ cm.} \end{array}$$

(2) For 40 gm of NaCl in cc of distilled water

$$\begin{array}{ll} n_1 = 2 & n_2 = 5 \\ r_1' = 1.2 \text{ cm.} & r_2' = 2.8 \text{ cm.} \end{array}$$

On substituting these values in the above equation we get

$$\frac{\mu'}{\mu} = 1.085$$

This result appears to be in the order of the values expected as the value obtained with Jamin's interferometer is 1.078.

This method is applicable not only to the solutions of different concentrations but also to gases of different pressures and temperatures. The advantage of this method lies in its applicability to the dynamically varying situations using pulsed laser.

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## ANNEALING CHARACTERISTICS OF INDUCED FISSION TRACKS IN MICACEOUS MINERALS

THE presence of uranium (even traces) in all the minerals is the basis of fission track technique used by Price *et al.*<sup>1</sup> for dating of mineral rocks and pegmatites<sup>2</sup>. Due to the effect of thermal annealing over the long periods of mineral history the ages determined by fossil fission track technique have been observed<sup>3</sup> to be lower than the corresponding ages determined by radiometric methods<sup>4,5</sup>. This work confines to the study of annealing effects observed at laboratory temperatures in micaceous minerals of Kodarma zone, Bihar mica belt.

There is no theory which can explain perfectly the annealing of radiation damage and rejuvenation of minerals on heating to various temperatures. However, some models have been proposed on the basis of experimental results<sup>6</sup>. According to one such model : when the mineral is heated to high temperatures the redistribution of atoms in the region affected by the ionising fission fragments takes place and any sort of strain present due to the radiation damage disappears. The degree of this annealing effect depends upon the temperature to which the sample is heated and the time of heating. For each mineral there is a characteristic high temperature at which the effect of strain is removed totally and the complete rejuvenation of it takes place. This temperature is known as annealing temperature.

The results of high temperature annealing experiments<sup>7</sup> in synthetic mica have shown that tracks formed nucleation sites for the decomposition of this material. On heating, the portion of the tracks in the interior of the crystals became narrower and eventually disappear.

It is clear from this discussion that the possibility of annealing of radiation damage and of track fading in minerals always exists. The track fading can be caused by a short time, high temperature event or by a long time anneal at a slightly elevated temperature during the geothermal history of earth's crust.

The minerals selected for annealing study are muscovite and biotite samples collected from Kodarma zone of Bihar mica belt. The age of the minerals has been estimated in a previous study<sup>2,3</sup>.

The range of each fossil fission track was determined by recording its projection along a calibrated microscope scale and dip in the sample by a fine motion Z screw of a Leitz binocular microscope using a magnification of 600 $\times$ . More than 500 tracks were measured in each sample. The mean range of fission tracks has been observed to be 18.5  $\mu$  and 15  $\mu$  in muscovite and biotite, respectively.

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The procedure adopted for the experimental study of annealing effects is the same as reported by Mehta and Rama<sup>8</sup>. It has been observed that the track length and the density of tracks remain unaltered at 100°C and 200°C in both the samples. Prolonged heating at 300°C reduces the track length slightly (18.2  $\mu$ ) in muscovite but the effect is quite appreciable in case of biotite (13.5  $\mu$ ). At 400°C, the track fading is more intense but at 500°C the tracks completely fade out in biotite samples for prolonged heating (6 hours) while in case of muscovite samples their density is reduced by 10%. On further heating of muscovite samples to 700°C it has been found that cylindrical tracks fade out into small distorted circular etch pits. This stage corresponds to nucleation phase change in mica<sup>7</sup>.

It was originally thought by Price *et al.*<sup>9</sup> that the fading of tracks during observation in the electron microscope was due simply to the warming of the samples by the electron beam. However, tracks in synthetic mica showed no fading in the microscope. Laboratory experiments have been done by various groups<sup>10-12</sup> to determine the annealing temperatures of various minerals to unravel the geothermal history of earth's crust. Our results are summarized as follows:

(1) Both muscovite and biotite samples remain unaffected at low temperatures and the reduction in track density is so small that the age correction may be neglected.

(2) The annealing temperature for muscovite is 700°C and for biotite it is 500°C. Thus our result is in agreement with that reported by Mehta and Rama<sup>8</sup>.

(3) The small difference observed in average fossil track length and the induced track length in micaceous minerals indicates that there has been no major orogenic metamorphic cycle in the geothermal history of the region from where the samples were collected.

Department of Physics,  
Punjabi University,  
Patiala (India), October 26, 1974.

H. S. VIRK.  
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## PARAQUAT-CALCIUM ADSORPTION EQUILIBRIUM IN SOIL CLAYS

PARAQUAT (1 : 1'-dimethyl-4 : 4'-dipyridylum dichloride) a divalent organic cation, a weedicide and a defoliant, is quickly deactivated due to strong adsorption on soil colloids. Not all the adsorbed paraquat is converted to the herbicidally inactive form but a portion goes into the non-exchangeable form and loses its bio-activity<sup>1</sup>. As a cation, this herbicide is known to get preferentially adsorbed on soils, in preference to ammonium<sup>2</sup>. However, in the soil,  $\text{NH}_4^+$  is only a minor exchangeable ion, but it is  $\text{Ca}^{2+}$  which forms a major portion of the exchangeable cations in many soils. The adsorption behaviour of paraquat<sup>2+</sup> in the presence of  $\text{Ca}^{2+}$  is not known. In order to find out the presence of these two ions on clays, paraquat<sup>2+</sup> -  $\text{Ca}^{2+}$  equilibrium studies were carried out on clay fractions isolated from red, laterite, black and *karl* soils, following the methods of Vansant and Uytterhoeven<sup>3</sup>. Mineralogically red and laterite soil clays are dominantly kaolinitic and black and *karl* soil clays are dominantly montmorillonitic. Paraquat<sup>2+</sup> was determined by alkaline dithionite method<sup>4</sup> and  $\text{Ca}^{2+}$  by titrating against standard versenate.

The results reveal that larger quantities of  $\text{Ca}^{2+}$  are adsorbed on kaolinite-dominant clays than on montmorillonite-dominant clays, while paraquat<sup>2+</sup> is preferentially adsorbed on montmorillonitic clays. It is expected that in the expanding montmorillonitic clays, the organic cation is fixed, in between the clay unit cells in a non-exchangeable form, unlike in the non-expanding kaolinitic clays. The size of paraquat ion has been shown to be almost the same as the inter-layer space of montmorillonite<sup>2</sup>, which facilitates the specific adsorption of the cation in such clays.

This is further confirmed by the values of  $\Delta G$  (adsorption) obtained from the equilibrium values of the reaction  $\text{paraquat}^{2+} \rightarrow \text{Ca}^{2+}$ . These values were calculated from the concentrations of  $\text{Ca}^{2+}$  and paraquat<sup>2+</sup> in the equilibrium solution and the quantities of these ions adsorbed on the clays as per the method of Vansant and Uytterhoeven<sup>3</sup>. On red and laterite soil clays the  $\Delta G$  values are

+953.8 cal/equiv. and +798.9 cal/equiv. respectively, whereas those on the black and *karl* soils the  $\Delta G$  values are -53.7 cal/equiv. and -59.6 cal/equiv. respectively. These indicate the preference of  $\text{Ca}^{2+}$  on red and laterite soils and paraquat<sup>2+</sup> on black and *karl* soils. Hence, deactivation of the weedicide is higher in black and *karl* soils than in red and laterite soils.

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Agricultural College, Hebbal,  
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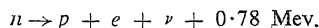
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#### PRIMORDIAL NUCLEAR MATTER AND NUCLEOSYNTHESIS

MANY theories have been proposed to explain the observed relative abundance of elements in the universe particularly in the solar system where experimental data are easily available. None of these theories is in a position to explain all the features of the abundance curve<sup>1</sup>. The latest approach made by Burbidge *et al.*<sup>2</sup> introduces eight different processes to create elements to cover the entire mass region (1 to 260). The fundamental question about the starting material however remains to be settled. The failure of  $\alpha, \beta, \gamma$  theory which assumes neutrons as the primordial matter lead Burbidge *et al.* to assume that hydrogen was the starting material. In this note it is shown that neutron is a better choice and elements can be synthesized from neutron matter. More detailed results will appear elsewhere.

The well-known fact that our observable universe is made up of neutrons, protons and electrons leaves us with only two choices: neutron and hydrogen. A convincing proof about the neutron being the primordial matter comes from beta decay. In beta decay a neutron decays to proton an electron and a neutrino. It has been experimentally proved that the properties of beta particles emitted from a nucleus and those of the extranuclear electrons are same (same charge, mass, spin, etc.). This strongly suggests that they have the same origin. In other words electrons are merely the decay products of neutrons. If this is true, then neutrons must have existed in the prestellar conditions and must be taken as the primordial matter.

The decay of neutrons can be represented as



the decay energy of neutrons will raise the temperature of the mixture ( $n + p + e + \nu$ ) to a billion degree or more. When the proton density becomes high the reverse reaction  $n \leftarrow p + e + \nu$  will stop the neutrons from being destroyed. At this temperature fusion reactions will start to build up the heavy nuclei. Neutron capture will be another competing reaction which will contribute to element formation.

The hydrogen will be the product of a later stage when under suitable conditions protons and electrons combine to form a bound system.

Institute of Science, B. M. P. TRIVEDI.  
Bombay-32, India, October 5, 1974.

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#### A REINVESTIGATION OF IRON-RESACETOPHENONEOXIME COMPLEX : AN IMPROVED PROCEDURE FOR THE SPECTROPHOTOMETRIC DETERMINATION OF IRON (III)

IN our attempts to determine the stability constant of the iron-resacetophenoneoxime complex, we observed that iron (III) and resacetophenoneoxime give rise to a mixture of complexes in addition to the single species of 1:1 complex (pH 4.5 to 7.0) reported by Raja Reddy *et al.*<sup>1</sup>. They made use of the Hilger Spekker Absorptiometer with Ilford colour filters in their investigations. We have now made a detailed study using UVISPEK Photoelectric Spectrophotometer (Hilger and Watts Ltd., manual type).

The reagent has negligible absorbance in the spectral region studied, 400 to 650 nm. The absorption curves were drawn at different pH values; the measurements were made against the reagent blank in 10% v/v aqueous ethanol. The metal to reagent ratio was maintained at 1:50 because it was found that at lower concentrations such as 1:6 turbidity developed thereby causing difficulties for accurate measurements.

An isobestic point in the pH range 6.0 to 8.3 at 580 nm clearly indicated the presence of mixture of complexes. But in the pH range 2.5 to 5.0, the absorption curves do not pass through the isobestic point indicating the presence of a single species which is in confirmation with the results reported by Raja Reddy *et al.* at pH 4.5. At pH

values between 2.0 to 3.0 the colour of the complex is violet and fades away with time, while at higher pH values the colour is reddish-violet and stable for 24 hrs.

In view of these facts it is not possible to determine the stability constants by the Job's method of continuous variation. These studies, however, have revealed that there is no need at all for maintaining rigorous control of pH for the determination of Fe(III) when the measurements of the optical densities are made at 580 nm. We, therefore, recommend the following modified procedure for the determination of iron(III) with resacetophenoneoxime in 10% v/v ethanol-water medium.

An aliquot of iron(III) solution is treated with resacetophenoneoxime in ethanol in fiftyfold excess and the pH adjusted to 6.0 to 8.3 with dilute sodium hydroxide (0.01 M) and the absorbance measured at 580 nm. Beer's law is obeyed in the range 1-14 ppm.  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ,  $\text{Hg}^{++}$ ,  $\text{Cd}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{--}$  have no effect even when present in excess.  $\text{CH}_3\text{COO}^-$ ,  $\text{F}^-$ , and  $\text{Al}^{+++}$  are tolerable upto 50 ppm. Oxalate, citrate, borate and  $\text{Mn}^{++}$  interfere when the concentration exceeds 10 ppm. The tolerance of a given ion is a maximum amount that can produce an absorption difference of 3% from that of the iron complex (Fe: 5 ppm). Phosphate, carbonate and  $\text{Co}^{++}$  interfere seriously. Copper<sup>2+</sup>, nickel<sup>3</sup> and palladium<sup>1</sup> interfere as they produce precipitates with the reagent.

Department of Chemistry, G. S. CHOWDARY,  
Sri Venkateswara University, N. APPALARAJU.  
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### INFRA-RED CARBONYL FREQUENCIES OF HYDROXY CHALKONES AND CHROMONES

IN some of our earlier papers<sup>1-3</sup> on the effect of conjugation and hydrogen bonding on the infra-red carbonyl frequency, certain abnormal features were observed. There were special cases in which the introduction of a hydroxyl group, in a position that can cause chelation, raises unexpectedly the infra-red carbonyl frequency rather than lower it. This was attributed to the presence of a conjugated

—O—C=C—C=O system in which the ethylenic double bond was not a part of a benzenoid ring. Such an abnormal feature was first noticed by

comparing the infra-red spectra of 5-hydroxy-flavones and isoflavones on the one hand and the corresponding flavanone and isoflavanone derivatives on the other<sup>4-5</sup>. The former group of compounds shows this special effect whereas the latter group does not, obviously due to the absence of an ethylenic double bond in the oxygen ring. That the benzenoid double bond is not capable of bringing about such an effect, was established by the recent study of the I.R. spectra of suitably substituted hydroxy xanthenes, benzophenones and anthraquinones<sup>6</sup>.

In a further study discussed in this paper, we have examined compounds which have an ethylenic double bond but without oxygen linked to it, *i.e.*, chalkones (I). In them, the chelation of the ortho hydroxyl with the chalkone carbonyl brings about the normal lowering of the C=O frequency. When the chelation is removed by acetylation or methylation, the C=O frequency is increased. Thus 2'-hydroxy-4',6',4-trimethoxy chalkone absorbs at 1613  $\text{cm}^{-1}$ , while 2',4',6',4-tetramethoxy chalkone absorbs at 1648  $\text{cm}^{-1}$  and 2'-acetoxy-4',6',4-trimethoxy chalkone absorbs at 1652  $\text{cm}^{-1}$ . The same is the case with other chalkones (Table I). Thus chalkones behave similar to flavanones and isoflavanones as against flavones and isoflavones obviously due to the absence of ether oxygen as the electron source.

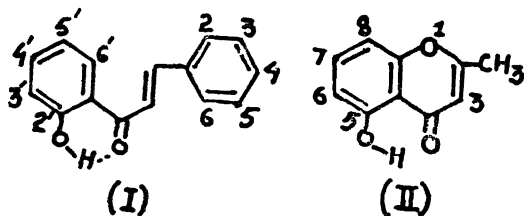


TABLE I  
Infra-red carbonyl frequencies of chalkones (I)

| Compound                                    | $\nu_{\text{max}}^{\text{KBr}}$ | C=O ( $\text{cm}^{-1}$ ) |
|---|---------------------------------|--------------------------|
| 1. Chalcone <sup>7</sup>                    | ..                              | 1659                     |
| 2. 2'-hydroxychalcone <sup>7</sup>          | ..                              | 1637                     |
| 3. 2'-methoxy <sup>7</sup>                  | ..                              | 1650                     |
| 4. 4'-hydroxy <sup>7</sup>                  | ..                              | 1653                     |
| 5. 4'-methoxy <sup>7</sup>                  | ..                              | 1656                     |
| 6. 2'-hydroxy-4'-methoxy <sup>8</sup>       | ..                              | 1625                     |
| 7. 2', 4'-dimethoxy <sup>8</sup>            | ..                              | 1650                     |
| 8. 2', 3, 4'-trihydroxy-                    | ..                              | 1621                     |
| 9. 2', 3, 4'-triaceoxy-                     | ..                              | 1661                     |
| 10. 2'-hydroxy-4', 4-dimethoxy <sup>8</sup> | ..                              | 1620                     |
| 11. 2'-acetoxy-4', 4-dimethoxy <sup>8</sup> | ..                              | 1652                     |
| 12. 2'-hydroxy-4', 6', 4-trimethoxy-        | ..                              | 1613                     |
| 13. 2'-acetoxy-4', 6', 4-trimethoxy-        | ..                              | 1652                     |
| 14. 2', 4', 6', 4-tetramethoxy-             | ..                              | 1648                     |
| 15. 2', 3', 4', 3, 4-pentahydroxy-          | ..                              | 1619                     |
| 16. 2', 3', 4', 3, 4-benzoyloxy-            | ..                              | 1656                     |

TABLE II

Infra-red carbonyl frequencies of chromones (II)

| Chromone                                 | $\nu_{\text{max}}^{\text{KBr}} (\text{C}=\text{O}) \text{ cm}^{-1}$ |
|--|---|
| 1. 5, 7-dihydroxy-2-methyl chromone      | 1660  |
| 2. 5, 7-diacetoxy-2-methyl chromone      | 1648  |
| 3. 5-hydroxy-7-methoxy-2-methyl chromone | 1658  |
| 4. 5-acetoxy-7-methoxy-2-methyl chromone | 1645  |

As representing a simpler structure analogous to flavones and isoflavones, a few chromones have been studied for their infra-red carbonyl frequency with and without chelation (Table II); here also, as expected, chelation brings about the special effect, i.e., the carbonyl frequency is increased when chelation is present. Hence the generalization regarding this unusual chelation effect has been further supported.

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Delhi 110 007, October 16, 1974. T. R. SESHADRI.

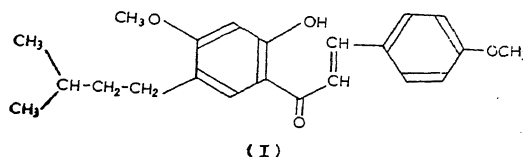
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### SYNTHESIS OF DERIVATIVES OF SOME NATURALLY OCCURRING CHALCONES

THE synthesis of the methyl ether of dihydro 'Bavachalcone', a naturally occurring chalcone isolated from the seeds of *Psoralea corylifolia* Linn.<sup>1</sup> and some chalcones allied to the chalcone 'Flemichapparin' isolated from *Flemingia chappar* Ham<sup>2</sup> have been described here.

4-Isoamylresorcinol<sup>3</sup> was synthesised by the Nencki reaction by heating resorcinol with isovaleric acid in presence of anhydrous zinc chloride.

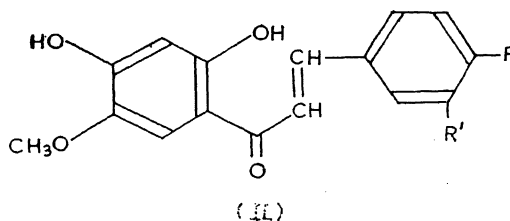
A Clemmensen reduction of the above ketone afforded 4-isoamylresorcinol<sup>4</sup>. A second Nencki reaction on the latter with acetic acid and anhydrous zinc chloride yielded 2, 4-dihydroxy-5-isoamylacetophenone as an oil (2, 4-DNP, m.p. 200°) which was partially methylated with methyl iodide in acetone solution to give 2-hydroxy-5-isoamyl-4-methoxyacetophenone as a colourless oil (2, 4-DNP, m.p. 206°). The latter when condensed with *p*-anisaldehyde gave orange-red plates of the methyl ether of dihydrobavachalcone (I) crystallised from dilute alcohol, m.p. 88–89° (Calcd. for  $\text{C}_{22}\text{H}_{26}\text{O}_4$ : C, 71.5; H, 7.3; Found: C, 71.1; H, 6.9%).



$\nu_{\text{max}}^{\text{CH}_2\text{Cl}_2}$  1630 (–CO–CH=CH–),  
2850 (–OCH<sub>3</sub>)  $\text{cm}^{-1}$ .

The synthetic route employed for the synthesis of chalcones allied to Flemichapparin is similar to that described by the earlier workers<sup>2</sup>. It however differs in the method of methylation and debenzoylation of the methylated product.

Resacetophenone-4-benzylether<sup>5</sup> on oxidation with potassium persulphate gave 4-benzoyloxy-2, 5-dihydroxyacetophenone, m.p. 158–61° (2, 4-DNP, m.p. 240–42°). Methylation of the latter with dimethyl sulphate in acetone solution was attempted but working out as usual and crystallization from ligroin afforded only 4-benzoyloxy-2, 5-dimethoxyacetophenone, m.p. 110–11° (2, 4-DNP, m.p. 176°). However, methylation with dimethylsulphate and NaOH yielded the 5-methyl ether, m.p. 128–29° (2, 4-DNP, m.p. 236–38°). The latter on debenzoylation with hydrochloric acid in acetic acid produced 2, 4-dihydroxy-5-methoxyacetophenone, m.p. 169–71° (2, 4 DNP, m.p. 288–9°). The debenzoylated compound on condensation with different aldehydes gave Flemichapparin IIa (identified by m.m.p. and superimposable IR) and the chalcones related to it having the formulae II b–II d.





II a : R = H, R' = H; m.p. 158-60°.

(Calcd. for  $C_{16}H_{11}O_4$ : C, 71.11; H, 5.18.

Found: C, 70.9; H, 5.0%.)

$\lambda_{\text{max}}^{\text{MeOH}}$  225 (log  $\epsilon$  4.07), 313 nm (log  $\epsilon$  4.32).

$\nu_{\text{max}}^{\text{KBr}}$  1640 (conjugated CO),  
3448 (phenolic OH)  $\text{cm}^{-1}$

II b : R =  $\text{OCH}_3$ , R' = H; m.p. 164-5°.

(Calcd. for  $C_{17}H_{13}O_5$ : C, 68.00; H, 5.33.

Found: C, 68.4; H, 5.7%.)

$\lambda_{\text{max}}^{\text{MeOH}}$  235 (log  $\epsilon$  4.09), 380 nm (log  $\epsilon$  4.32).

$\nu_{\text{max}}^{\text{KBr}}$  1640 ( $-\text{CO}-\text{CH}=\text{CH}-$ ),  
3390 (phenolic OH), 2857 ( $\text{OCH}_3$ )  $\text{cm}^{-1}$ .

II c : R =  $\text{OCH}_3$ , R' =  $\text{OCH}_3$ ; m.p. 150-1°.

(Calcd. for  $C_{18}H_{15}O_6$ : C, 65.45; H, 5.45.

Found: C, 65.8; H, 5.2%.)

$\lambda_{\text{max}}^{\text{MeOH}}$  261 (log  $\epsilon$  4.02), 385 nm (log  $\epsilon$  4.36).

$\nu_{\text{max}}^{\text{KBr}}$  1640 ( $-\text{CO}-\text{CH}=\text{CH}-$ ),  
3333 (phenolic OH),  
2857, 1250 ( $\text{OCH}_3$ )  $\text{cm}^{-1}$ .

II d : R, R' =  $-\text{O}-\text{CH}_2-\text{O}-$  (Methylenedioxy), m.p. 162-3°.

(Calcd. for  $C_{17}H_{13}O_6$ : C, 64.96; H, 4.45.

Found: C, 64.7; H, 4.6%.)

$\lambda_{\text{max}}^{\text{MeOH}}$  265 (log  $\epsilon$  4.01), 385 nm (log  $\epsilon$  4.32).

$\nu_{\text{max}}^{\text{KBr}}$  1640 ( $-\text{CO}-\text{CH}=\text{CH}-$ ),  
3425 (phenolic OH),  
930 ( $-\text{O}-\text{CH}_2-\text{O}-$ )  $\text{cm}^{-1}$ .

We are grateful to Dr. Adityachaudhury for an authentic sample of Flemichapparin and to Mrs. J. A. Patankar and Shri D. S. More for microanalyses.

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# INVERSE RELATIONSHIP BETWEEN PLASMATOCYTES AND ADIPOHAEMOCYTES OF *DYSDERCUS CINGULATUS* FABR. (HEMIPTERA : PYRRHOCORIDAE) RELATED TO AGE AND REPRODUCTIVE CYCLES

THE changes in the percentage of one or the other types of haemocytes during their life cycle in *Forficula auricularia*<sup>1</sup>, *Prodenia eridania*<sup>2</sup>, *Ephesia kühniella*<sup>3</sup>, *Rhodnius prolixus*<sup>4</sup>, *Galleria mellonella*<sup>5</sup> and *Drosophila euronotus*<sup>6</sup> have been recorded. The present information on *Dysdercus cingulatus* is related to the changes in the percentage of plasmatocytes and adipohaemocytes related to age and reproductive cycles of the adults.

The method for differential counts of these cells is based on that of Jones<sup>4</sup> with slight modification for counting various haemocytes under light microscope. For this purpose the blood smears were stained with Giemsa's stain. The confirmation of adipohaemocytes (haemocytes with fat droplets) was also made by using Sudan Black-B stain.

It was recorded that in both sexes of the newly emerged *D. cingulatus*, the percentage of adipohaemocytes is much higher than that of the plasmatocytes; and males have significantly higher percentage of adipohaemocytes than the females. The percentage of plasmatocytes in the newly emerged adults is higher in females than in the males (Fig. 1, A and B). Thereafter, in both sexes, the plasmatocytes increase in number. Following the emergence in contrast to the percentage of plasmatocytes, that of adipohaemocytes generally decreases with respect to advancing age of both sexes (Fig. 1, B). However, in males, the initial decrease in the number

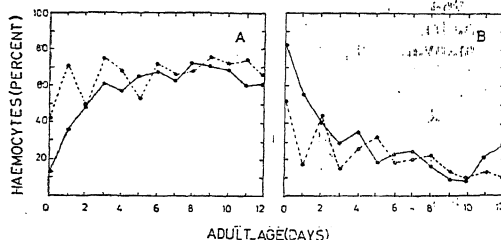


Fig. 1. A, Plasmatocytes, B, Adipohaemocytes.  
o—o—o—o—o Male  
o—o—o—o—o Female

of adipohaemocytes is more pronounced than in the females. In *D. cingulatus* at  $29^\circ\text{C} \pm 1^\circ\text{C}$  and 70%-80% R.H., the first batch of eggs is laid on 6th or 7th post-emergent day and then the second batch is oviposited on 10th or 11th day. Therefore, it is clear that the increase in the percentage of plasmatocytes and the decrease of the adipohaemocytes are quite significant before the first

reproductive cycle. Changes in the percentage of both types are slow and less marked.

From the present data on *D. cingulatus*, it is concluded that there is a distinct sexual difference in the population of plasmotocytes and adipohaemocytes which is not known in any insect species so far studied. Further, the latter type of haemocytes either degenerate or get consumed during the growth and metabolism of adults whereas, the former cells are produced in larger numbers. Such an inverse relationship between the plasmotocytes and adipohaemocytes has not been reported in any hemimetabolous insect. However, during the short adult life of *Ephestia kuehniella*<sup>3</sup> it is reported that plasmotocytes increase and spheroidocytes (loaded with fat globules) decrease.

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#### DIURNAL VARIATIONS IN ACETYLCHOLINE- ESTERASE ACTIVITY IN THE NERVOUS SYSTEM OF THE AESTIVATING SNAIL, *PILA GLOBOSA*

DESPITE the different types of rhythms reported in molluscs<sup>1</sup>, no work was done on the rhythms in enzyme activities. The snails are known to be active during nights<sup>2</sup>, and hence there might be variations in nervous activity in terms of acetylcholinesterase (AChE) activity during different periods of the day. The prosobranch snail *Pila globosa* aestivates during summer<sup>3</sup>. Since this dormancy involves several physiological changes on the part of the snail<sup>4</sup>, it would be interesting to study the diurnal variations in the AChE activity in the nervous system of normal, aestivated and revived snails, and also with reference to the effect of eserine, a known inhibitor of AChE<sup>5</sup>.

Normal snails were maintained in tap water in aquarium jars and fed with *Hydrilla*. Aestivation was induced in them by burying in dry sand in wooden boxes. Aestivated ones were revived by placing them in water in glass jars, and they were soon fed with *Hydrilla* and used one day after revival for experimentation.

For the assay of AChE activity, the nervous tissue, including the ganglia, connectives and com-

missures was isolated in cold from normal, aestivated and revived specimens separately. Six different timings, viz., 12 noon, 4 p.m., 8 p.m., 0 hrs, 4 a.m. and 8 a.m. were chosen to cover the 24-hour period of the day. Each time six samples were analysed. The experiment was repeated for three consecutive days to see whether the pattern of activity remained the same on all the three days. 5% (W/V) homogenates of the nervous tissue were prepared in 0.25 M sucrose solution. The homogenates were assayed for AChE activity, adopting the method of Metcalf (1951) as given by Glick (1957)<sup>6</sup> with due modifications to suit the present material. The enzyme activity was expressed as  $\mu$  moles of ACh hydrolysed/mg protein/hr.

For studying the effects of eserine, the incubation mixtures received 0.1  $\mu$  mole of eserine.

The results clearly show that a regular diurnal rhythm of AChE activity exists in the nervous system of *Pila globosa* (Fig. 1). An interesting

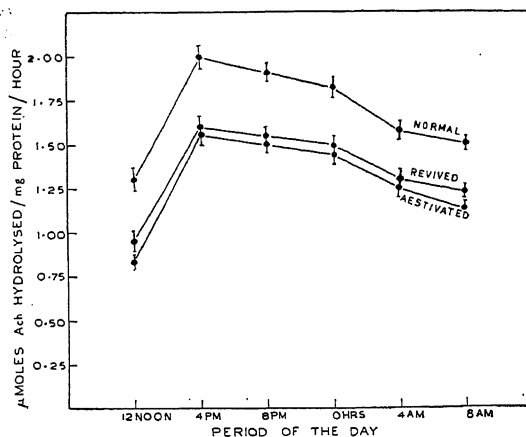


FIG. 1. AChE activity levels at different periods of the day in the nervous system of normal, aestivated and revived *Pila globosa*.

feature was that this rhythm was observed in the aestivated and revived specimens also, indicating that this is an endogenous activity and was not disturbed during aestivation. The activity was found to be maximum at 4 p.m. and thereafter it decreased gradually, reaching the minimum at 12 noon in all the specimens studied. A decrease in the enzyme activity was observed at all periods in the aestivated specimens as compared to the normals. Upon revival, the activity tended to recover towards the normal level (Fig. 1).

*Helix*, *Limax* and other snails have been reported to become active towards the night<sup>2</sup>; and thus a higher level of nervous activity could be expected during night time. Since AChE activity represents one of the several facets of neuronal activity, the

observed rhythm in AChE activity in the present investigation can be presumed to go hand in hand with the general state of activity of the animal. Cholinesterase activity rhythm of slightly different nature has been shown in the slug *Vaginulus*<sup>7</sup> wherein the maximum activity was at 4 p.m. and minimum activity at 0 hrs, and in the scorpion *Heterometrus*<sup>8</sup> wherein the maximum and minimum activities were at 4 p.m. and 4 a.m. respectively.

Murali Mohan and Muralikrishna Dass (1969)<sup>9</sup> reported a decrease in AChE activity of the nervous system during aestivation of *Pila globosa*. In the present study, while maintaining the rhythm of AChE activity as in the normal snail, the aestivated snail showed a general decrease in the AChE activity at all the periods of the day in comparison to the normal snails. This indicates that the decrease in AChE activity is explicit during aestivation in the nervous system of the snail.

Eserine inhibited the AChE activity at all periods in normal, aestivated and revived snails, with the inhibition being greater in lower activity periods and *vice versa* (Fig. 2). This shows that the snail

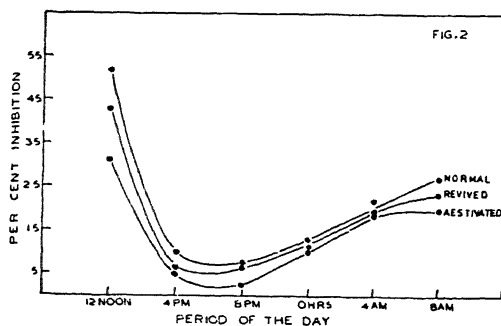


FIG. 2. Per cent inhibition of AChE by eserine at different periods of the day in the nervous system of normal, aestivated and revived *Pila globosa*.

is able to overcome the inhibitory effect to a large extent during the periods of higher activity. It may be hypothesized that the enzyme is subjected to masking and unmasking effects during low and high activity periods respectively, resulting in respective inhibition and activation during those periods. These possible masking and unmasking interactions might accordingly facilitate or antagonize the inhibitory influence of the effector added.

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#### BLOOD VESSELS AND CAPILLARIES OF NORMAL AND LEUCODERMIC SKINS

In the melanocytes of the normally pigmented skin, the enzyme tyrosinase/dopa oxidase converts dopa to melanin. For the activity of this enzyme molecular oxygen is necessary<sup>1</sup>. Molecular oxygen reaches these melanocytes through haemoglobin *via* blood. Therefore the blood vessels and capillaries of the skin are of vital importance for normal skin pigmentation<sup>2</sup>. According to Becker<sup>3</sup>, many authors believe that in vitiligo/leucoderma an impairment in vascularity of the skin is present. We have therefore investigated the blood supply system of the normal and leucodermic skins.

Leucodermic and the corresponding normal human skin were obtained by punch biopsy (4 mm) after local anaesthesia. They were blotted free of blood and 10  $\mu$  sections were cut on a freezing microtome. These sections were then stained for alkaline phosphatase activity<sup>4</sup>, which selectively stains the blood vessels and capillaries in the skin<sup>5</sup>.

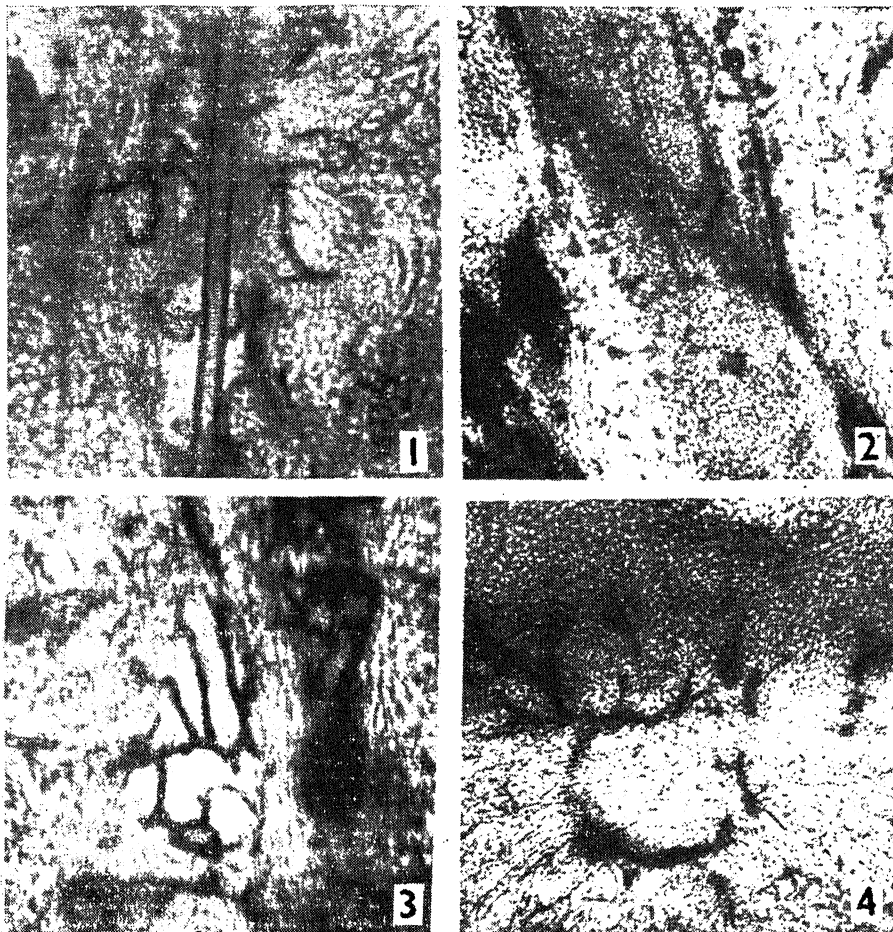
All the major blood vessels and capillaries could be identified in both the normal and leucodermic skins (Figs. 1-4). Since the alkaline phosphatase activity was normal, leucodermic skin does not represent traumatized skin<sup>5</sup>.

We<sup>6</sup> have recently shown that leucodermic skin can peroxidatively convert tyrosine to dopa, but is incapable of converting dopa to melanin *via* tyrosinase. As mentioned earlier molecular oxygen is necessary for this conversion. Since the blood supply and consequently oxygen supply to leucodermic skin is normal (Figs. 1-4) the cause for amelanogenesis lies elsewhere. It would be either due to the absence of the enzyme tyrosinase in leucodermic skin as it is undetectable by histochemical methods<sup>7</sup>, or it could be due to the presence of some antioxidant in the melanocytes which competes with tyrosinase for oxygen. One such antioxidant widely used in industry, viz., monobenzylether of hydroquinone has been identi-

fied by Oliver *et al.*<sup>8</sup> as the cause in certain types of occupational leucoderma.

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FIGS. 1-4. Fig. 1. Normal human skin showing the blood vessels surrounding the hair,  $\times 150$ , Fig. 2. Normal human skin showing the blood vessels in the skin. The darkly stained body is the highly alkaline phosphatase positive sebaceous glands,  $\times 150$ . Fig. 3. Leucodermic skin showing the rich network of blood vessels in the hair follicle,  $\times 150$ . Fig. 4. Leucodermic skin showing the blood vessels of the epidermis and dermis. Note the intense anastomosis of the blood vessels and capillaries,  $\times 150$ .

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# DISTRIBUTION AND ABUNDANCE OF THE PLANKTONIC SHRIMP, LUCIFER, IN THE NEARSHORE WATERS OFF VISAKHAPATNAM (BAY OF BENGAL)

The planktonic shrimp belonging to the genus *Lucifer* is a very common constituent of the zooplankton in the seas around India. Three species, *L. penicillifer*, *L. hanseini* and *L. typus* occur in the nearshore waters off Visakhapatnam, of which *L. penicillifer* contributes to the bulk of the lucifers. The present paper reports the seasonal abundance and distribution of the lucifers in relation to the prevailing hydrographical conditions.

Samples of zooplankton were collected from March 1968 to February 1970 with an International Coarse Silk Net (mesh size 0.28 mm) by vertical hauls from a depth of 40 metres. The developmental stages of all the three species were enumerated together, owing to the absence of well marked features differentiating them, while the adults were sorted out species-wise, and counted separately.

The protozoa showed a major peak in June and two minor peaks, one in April and another in December, 1968. During 1969 the major peak was in September, and two minor peaks, one in March and the other in December. The peaks of the juveniles more or less followed the same pattern as the protozoa, during both the years. The adults were present practically throughout the year. Numerically they were smaller than the developmental stages but at no time totally absent except in June 1969. The protozoa ranged from 0.02% to 3.13% of the total zooplankton in March and June 1968; the juveniles from 0.03% to 2.04% in April and October 1968 and the adults from 0.01% to 0.64% in April and October 1968. Numerically the protozoa were at their maximum (35 m<sup>3</sup>) in June 1968 and minimum (0.37/m<sup>3</sup>) in July 1968; the juveniles at their maximum (26.59 m<sup>3</sup>) in March 1969 and minimum (0.16/m<sup>3</sup>) in January 1970 and the adults at their maximum (2.81 m<sup>3</sup>) in November 1969 and minimum (0.16/m<sup>3</sup>) in August 1968. The major peaks of the developmental stages were observed in relatively high saline waters of the Northerly Current (February-June) and minor peaks during the low saline Southerly Current period (October-December). No appreciable difference in the numerical abundance of the adults was observed during the two current periods. It would also appear that there are at least 4-5 broods in a year.

Of the three species, *L. penicillifer* was the most common and the dominant species. *L. hanseini* appeared in smaller numbers throughout the period

of investigation. *L. penicillifer* is a well-known inhabitant of the coastal waters in the Indo-Pacific region. *L. typus* appeared in the coastal waters in large numbers from February to June during the Northerly Current period along with the incursion of the oceanic waters into the coastal region. The preliminary observations we have made from samples collected during a cruise (April-May 1969) to the Andaman Islands, Bay of Bengal (Visakhapatnam to Port Blair via Madras) also revealed the same pattern of distribution. *L. penicillifer* was abundantly present in the samples collected within 100 km distance from the coast. Large numbers of *L. typus* were present in waters beyond the 100 km zone. The pattern of distribution of the three species clearly shows that *L. typus* is a true oceanic species, which occasionally appears in the nearshore region along with the incursion of the offshore and oceanic waters into the coastal region during the Northerly Current period. Wickstead<sup>1</sup> and Bowman and McCain<sup>2</sup> also observed *L. typus* as an oceanic species in the Singapore Strait and Western North Atlantic respectively. *L. typus* is thus a good practical indicator of offshore and oceanic waters incursion into the nearshore region off Visakhapatnam.

One of us (K. V. R.) thank the C.S.I.R. for the grant of a Research Fellowship during the tenure of which the present work was undertaken. We also thank Professor K. Hanumantha Rao, Head of the Department of Zoology, for providing all facilities and encouragement.

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## A NEW SPECIES OF SPIDER OF THE GENUS *PEUCETIA* THORELL (FAMILY OXYOPIDAE) FROM ORISSA, INDIA

THE genus *Peucetia* Thorell of the family Oxyopidae appears to be little known from India. The first species was described from India by Stoliczka (1869) and subsequently Pocock (1900) described three species. Recently Tikader (1965, 1970) has described two species of the genus *peucetia* from India. While examining the spider collection from Orissa (India) the following new species was noticed. The type specimen has been deposited in the Zoological Survey of India, Calcutta.

The author's sincere thanks are due to the Director, Zoological Survey of India, for giving

necessary facilities and to Dr. J. K. Sen for encouragement for this work. He is indebted to Dr. B. K. Tikader, Deputy Director, Zoological Survey of India, Western Regional Station, Poona, for guidance and verification of his identification and help in the preparation of this paper.

*Peuceetia harishankarensis* Sp. Nov.

**General:** Cephalothorax light green, legs reddish brown, abdomen magenta colour. Total length 17.00 mm. Carapace 6.00 mm long, 4.50 mm wide; abdomen 11.00 mm long, 5.55 mm wide.

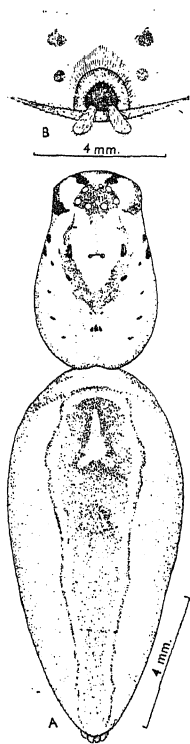


FIG. 1. A-B. *Peuceetia harishankarensis* sp. nov. A, Dorsal view of female, legs omitted; B, Epigyne.

**Cephalothorax:** Longer than wide, narrow in front and provided with conspicuous black spots and U-shaped red marking. Cephalic region high. Eyes eight and situated on the elevated portion of the cephalic region and all eyes encircled by black patch. Posterior row slightly procurved and equidistant, anterior row recurved and anterior medians very small. Clypeus long and broad provided with a pair of black lines extending from ocular area to the base of the fang. Sternum oval; clothed with fine hairs. Legs long and strong with conspicuous long spines.

**Abdomen:** Long, narrowing behind, clothed with hairs. Middorsally provided with a longitudinal

broad olive green band extending from base to the end of abdomen, as in Fig. 1. Ventral side light colour. Epigyne as in Fig. 1B.

**Type Specimen:** One female in spirit (HOLOTYPE).

**Type-locality:** Gandhamardan Hill (3,090 feet) near Harishankar, Bolangir, Orissa, India. Coll. Dr. J. K. Sen. 3-11-1973.

This species resembles *Peuceetia viridans* (Heritz.) but differs as follows: (i) Cephalothorax with black spots but in *P. viridans* cephalothorax without black spots. (ii) Epigyne also structurally different.

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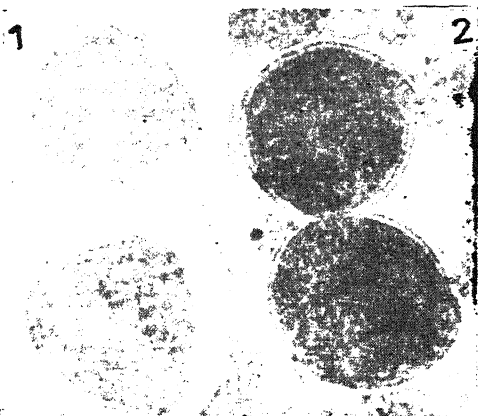
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# A NEW REPORT ON WHITE FLOWERS IN *TEPHROSIA PURPUREA* PERS. COMPLEX

*Tephrosia purpurea* is reported to be polymorphic species as observed in the populations growing at Waltair<sup>1</sup>. This feature is also shown in populations of this species in most other countries in which it occurs. The same is true for *T. purpurea* at Jodhpur. Two populations: (1) procumbent showing *gigas* vegetative features; and (2) erect with smaller-sized vegetative parts and pods have been noted<sup>1</sup>.

As far as the information of the author goes, this is the first report of *T. purpurea* having pure white flowers. The colours of flower in populations range from crimson-red, red, pink, light pink, pinkish-white and pure white. The occurrence of plants having white flowers was first observed by the author in 1973 but their number appeared to increase in 1974. However, the white colour of petals still remains very rare. After measurements of different vegetative and floral characters of pink and white flowered plants, no distinctive differences were found. The major distinction was observed in pollen grains. The pollen grains when mature were found distinctly triangular in pink-flowered stamens (Fig. 1); whereas those in white-flowered ones, they were just circular (Fig. 2). However, the distinct red-coloured flowers sometimes possessed some roundish pollen grains as well. There appears to be some distinct difference of pollen grains and seed coat colours and their mottling in populations of *T. purpurea*.

Ramanathan<sup>2</sup> reported a chromosome number of  $2n=24$ ; whereas Tandon and Malik<sup>3</sup> observed  $n=11$  in *T. purpurea*. The latter number is confirmed by Venkateswarlu and Rao<sup>4</sup> for this species. Naturally occurring allotetraploid races have already been reported<sup>2,4</sup>.



FIGS. 1-2. Triangular pollen grains in pink-flowered plants of *T. purpurea*,  $\times 1,280$ . Fig. 2. Circular pollen grains in white-flowered plants of *T. purpurea*,  $\times 1,280$ .

This discovery of white coloured flowers in *T. purpurea* complex is noteworthy and its cytological study might reveal interesting results in its populations.

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#### EFFECT OF DIFFERENT NUTRIENT MEDIA ON THE GROWTH AND SPORULATION OF *USTILAGINOIDEA VIRENS* (CKE) TAKAHASHI

*Ustilaginoidea virens* causing false smut of rice was first reported from Tinnevely in Tamil Nadu State by Cooke<sup>1</sup>. Since then it has been reported from various parts of the world like Japan, U.S.A., etc. In the year 1973, it appeared in an epidemic

form in some areas at Jabalpur. The disease being of considerable economic importance, it was necessary to carry out certain physiological studies on the fungus.

The present paper deals with the effect of different nutrient media. The development of a suitable medium for growth and sporulation would facilitate studies of factors affecting the formation of metabolites in pure culture. From a practical standpoint, a medium capable of stimulating sporulation (conidial) of the fungus would aid in the preparation of spore suspensions required for inoculation of large populations in disease resistance breeding programs and in studies on the nature of disease resistance. Pure culture of *U. virens* was obtained by the following method.

Fresh sclerotia of false smut of rice (Fig. 1) collected from the field during 1973-74 crop from



FIG. 1. False smut of Rice: Infected earhead.

Agricultural Experimental fields, Adhartal, Jabalpur, were used for the present study. For obtaining axenic culture a sterilized coverslip was put in the centre of a petridish containing yeast-peptone-potato-dextrose-agar medium (YPPDA yeast 100 mg; peptone

TABLE I

*Dry weight, colony character and sclerotial formation of U. virens on different nutrient media (after 45 days of incubation at 23° C)*

| Medium                        |    | Dry weight (mg) | Colony color           | Sclerotial formation with conidia |
|-------------------------------|----|-----------------|------------------------|-----------------------------------|
| Potato-dextrose broth         | .. | 45.0            | White later greenish   | Moderate                          |
| PD broth + yeast + peptone    | .. | 181.0           | White later brownish   | Excellent                         |
| Rice meal-dextrose            | .. | 83.0            | White later yellowish  | Poor                              |
| Oat meal-dextrose             | .. | 13.0            | Greenish white         | ..                                |
| Corn meal-dextrose            | .. | 46.0            | White later dark green | Moderate                          |
| Richards' medium              | .. | 29.0            | Greenish-brown         | ..                                |
| Czapek's medium               | .. | 23.0            | Gelatinous             | ..                                |
| Brown's medium                | .. | Traces          | Gelatinous             | ..                                |
| Asthana and Hawker's medium   | .. | 10.0            | Gelatinous             | ..                                |
| Sartoris medium               | .. | 36.0            | Yellowish-green        | Poor                              |
| Yeast extract-dextrose medium | .. | 14.0            | Gelatinous             | ..                                |

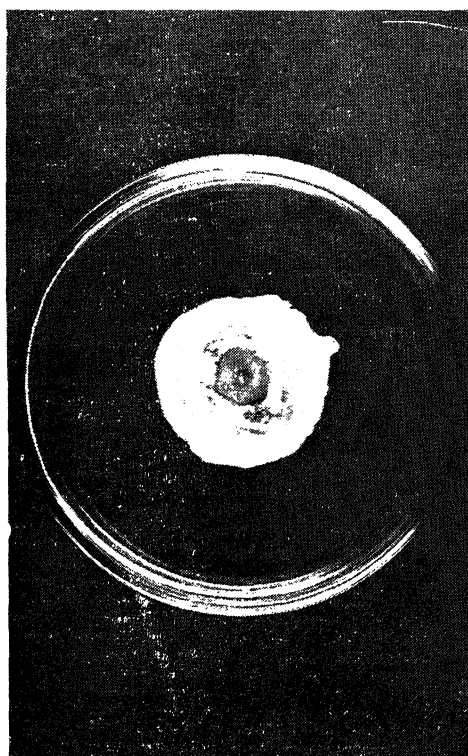


Fig. 2. Growth and conidial (sclerotial) formation on YPPDA medium.

100 mg; potato 200 g; dextrose 20 g; agar 20 g and distilled water 1000 ml). The conidia collected from fresh sclerotial balls were dusted over it. A drop of sterilized distilled water was placed at the border of the coverslip at two places and then incubated at 23° C spores germinated within 24 hrs and catches the medium. Clusters of 'sporidia' or secondary conidia were formed at the hyphal tip. Sometimes the growth of hyphae was slow for that 4-5 ovaries of susceptible variety of paddy flowers removed aseptically, placed over the coverslip and crushed in distilled water. This accelerates that growth of germtube of the germinating spore. When considerable development of the hyphae from the germinating spores occurs, it is removed aseptically and placed in the petridish containing YPPDA medium. A dense white aerial mycelium was formed, which produced large, round masses (sclerotia) in about a month (Fig. 2). These were first white, compact and almost round; later on they became orange-yellow and finally olive-green and powdery. Spores are produced in abundance from sclerotia, usually round to elliptical, smooth, paler, 3.5 to 5.5  $\mu$  in diam.

Cultures were made by taking 50 ml of the different media in 250 ml Erlenmeyer flasks and sterilizing in an autoclave at 15 lb (121° C) for 15 minutes. The flasks were then inoculated with equal amounts of inoculum and incubated at 23° C for 45 days. At the end of incubation period,



the dry weights of mycelial mats and sporulation (sclerotia with conidia) were recorded.

It is clear from Table I that *U. virens* gives best dry weight on potato-dextrose yeast-peptone medium followed by rice meal, corn meal, potato-dextrose, Santori's, Richards' and Czapek's medium. Growth was scanty on Ashana and Hawker's and Brown's medium. It seems that yeast and peptone in the natural medium (PD broth) exerts a dominating influence on the mycelial growth of the fungus.

Sclerotial formation was excellent on potato-dextrose-yeast-peptone medium. These were at first white and compact and almost round; later they became orange-yellow and finally olive-green and powdery due to abundant conidial production as observed by Seth<sup>2</sup> and Su<sup>3</sup> on Quaker oat medium. It was moderate on potato-dextrose broth and corn meal and poor on rice meal medium. In the present investigation potato-dextrose-yeast-peptone was found to be the best nutrient medium for the growth and conidial production of *U. virens*.

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#### SUPPRESSION OF *VERTICILLIUM* WILT BY *RHIZOCTONIA* ROOT ROT OF COTTON

THE wilt (*Verticillium dahliae* Kleb.) and the root rot (*Rhizoctonia solani* Kühn) of cotton were not observed to occur together in the same patch of the field, and in the earlier observed 'wilt sick' patches where root rot had newly appeared, a decline in the incidence of wilt was noticed. Isolates of *R. solani* obtained from cotton roots were found to vary in virulence, some attacking the main roots and rapidly bringing about death of the plants (virulent), while others brought about partial destruction of rootlets and stunting of inoculated plants, but not their death (moderately virulent). In order to examine the possibility of an antagonistic relationship between the wilt and root rot pathogens, soil-plant cultures of the two pathogens were incorporated in soil held in pots at the rate of 1% by weight of unsterilized garden soil and the susceptible cotton variety MCU-5 (*Gossypium hirsutum*) was raised thereon. One of the virulent isolates of *V. dahliae* and two isolates of *R. solani* differing in virulence were used. The results are given in Table 1.

TABLE I  
Incidence of wilt and root rot in the presence  
of the two pathogens

| Inoculum                                  | No. of plants<br>affected<br>out of 20 |          |
|---|--|----------|
|   | Wilt                                   | Root rot |
| <i>V. dahliae</i>                         | .. 20                                  | ..       |
| <i>R. solani</i> (virulent)               | .. ..                                  | 19*      |
| <i>R. solani</i> (moderately virulent)    | .. ..                                  | 16†      |
| <i>V. dahliae</i> + <i>R. solani</i> (v)  | .. 1                                   | 17*      |
| <i>V. dahliae</i> + <i>R. solani</i> (mv) | .. 3                                   | 15†      |

\* Plants killed; † Plants not killed, but stunted.

While the presence of *R. solani*, either of the virulent or of the moderately virulent type, inhibited the incidence of wilt, the presence of *V. dahliae* did not appear to influence the incidence of root rot. Isolations made from roots and stems failed to yield cultures of *V. dahliae* indicating that invasion of the vascular system by the wilt pathogen had been prevented in the presence of *R. solani*. Similar results were obtained when "wilt sick" soil was infested with *Rhizoctonia* inoculum and also when inocula were applied to the roots by the 'root dip' technique using suspensions of conidia and mycelia respectively of the wilt and root rot pathogens.

The chemical PCNB which is specific for the control of *Rhizoctonia* was applied to patches of fields where stray incidence of wilt was noticed but root rot occurred on a large scale. It was applied as 'brassicol' at the rate of 10 kg per acre and well mixed with the top 9 inches of soil. The incidence of root rot decreased from 80 to 85% in the untreated spots, to 10 to 15% in treated spots, while the incidence of wilt increased from 3% to 33 to 45%.

In culture, *R. solani* grew much faster than *V. dahliae* but no evidence of mycelial invasion of *Verticillium* or of the elaboration of an antibiotic substance was obtained. *In vivo* also *R. solani* rapidly invaded cotton roots bringing about destruction of root tips and maceration of cortical tissue within 24 hours of inoculation. On the other hand, according to Garber and Houston<sup>1</sup>, *V. dahliae* takes at least 3 days for effecting entry into the cortical tissue of the root and reaching the vascular system. Thus it appears that *R. solani* achieves a pre-emptive seizure and destruction of the portals of entry and blocks infection by *V. dahliae*.

The use of PCNB for controlling root rot has to be viewed with caution and should be precluded from areas with a hazard of *Verticillium* wilt.

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# EFFECT OF DOWPON ON THE CONTENT OF PROTEIN AND PROTEOLYTIC ACTIVITY IN *CYNODON DACTYLON* PERS.

DOWPON (2,2-dichloropropionic acid) being a propionic acid compound, its mode of action differs from that of 2,4-D and other triazine compounds in several respects. Redemann and Hamaker<sup>1</sup>, and Choudhri<sup>2</sup> reported that dalapon is an efficient protein precipitant. Foy<sup>3</sup> has shown that dalapon is a fairly strong acid and a protein precipitant. Four concentrations of dowpon were used and the protein content and proteolytic activity<sup>4</sup> in the foliage were estimated at four intervals after application (Table I). The results show that the medium

the enzyme complex generally. The proteolytic activity of the enzyme is phenomenally increased by the herbicide at the concentration employed resulting in the precipitation of protein. The mode of action is primarily on immobilisation of protein an index of toxicity of the herbicide.

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TABLE I

*The effect of dowpon on protein content and proteolytic activity of the foliage of Cynodon dactylon*

| Treatment<br>Dowpon<br>kg/ha<br>No.    | 4         | 8         | 12        | 16        | Interval<br>(days) |
|--|-----------|-----------|-----------|-----------|--------------------|
| (Percentage of the protein in foliage) |           |           |           |           |                    |
| Control                                | 10 (0.20) | 11 (0.20) | 7 (0.22)  | 10 (0.20) |                    |
| 5                                      | 10 (0.20) | 9 (0.21)  | 6 (0.23)  | 8 (0.20)  |                    |
| 10                                     | 12 (0.21) | 9 (0.24)  | 11 (0.27) | 12 (0.21) |                    |
| 15                                     | 5 (0.27)  | 9 (0.25)  | 5 (0.34)  | 5 (0.21)  |                    |
| 20                                     | 2 (0.24)  | 4 (0.26)  | 5 (0.39)  | 2 (0.23)  |                    |

The values in parenthesis are the activities in micrograms/g.

concentration (10 kg/ha) increased the protein content slightly at the end of 4 days ; but 8 days after the spray all the concentrations reduced the protein content throughout.

In general, the proteolytic activity increases with an increase in the concentration of the herbicide for a given interval of time. Similarly the activity of a given concentration increases with the period upto 12 days and then there is a fall.

The proteolytic enzyme generally hydrolyses the protein molecule into other fractions of nitrogen. Higher activity of this enzyme results in greater hydrolysis of protein. Freiberg and Clark<sup>5</sup> recorded a similar observation in stem and shoots of soyabean using 2, 4-D. Leisure<sup>6</sup> stated that the effect of dalapon as a protein precipitant would inactivate

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## AN UNREPORTED LINKAGE GROUP IN RICE (*ORYZA SATIVA* L.)

INHERITANCE of purple pigment in coleoptile, auricle and glume was studied in a rice cross between T-141, a variety developed by the Orissa State Department of Agriculture and K-44-1 an improved scented variety of the Karnataka State. F<sub>1</sub> showed the presence of purple pigment in these parts and F<sub>2</sub> population consisting of 2,624 individuals was studied (Table I). F<sub>3</sub> behaviour confirmed the F<sub>2</sub> ratios. Joint segregation of these characters is presented in Table II.

TABLE I  
Character expression of parents,  $F_1$  and  $F_2$  segregation in T-141 and K-44-1

| Pigment in:   | T-141            | K-44-1             | F <sub>1</sub>     | F <sub>2</sub> segregation |                           |                 | X <sup>2</sup> |
|---------------|------------------|--------------------|--------------------|----------------------------|---------------------------|-----------------|----------------|
|               |                  |                    |                    | Ratio                      | Purple                    | White           |                |
|               |                  |                    |                    | Purple: White              |                           |                 |                |
| Coleoptile .. | White            | White              | Purple             | 39:25                      | Obs. 1585<br>Exp. 1599.00 | 1039<br>1025.00 | 0.3137         |
| Auricle ..    | White            | White              | Purple             | 387:637                    | Obs. 1010<br>Exp. 991.68  | 1614<br>1632.32 | 0.5440         |
| Glume ..      | Ripening<br>Gold | Ripening<br>Purple | Ripening<br>Purple | 117:139                    | Obs. 1230<br>Exp. 1199.25 | 1394<br>1424.75 | 1.4520         |

TABLE II  
Joint segregation of the characters in  $F_2$  in T-141  $\times$  K-44-1

| Characters   |    |                                       | Observed segregation |        |        |         | $X^2$   |
|--|----|---------------------------------------|----------------------|--------|--------|---------|---------|
|  |    |                                       | PP                   | PW     | WP     | WW      |         |
| Coleoptile (39:25) with:<br>(i) Auricle (387:637)<br>(one gene common) | .. | Observed                              | 911                  | 674    | 799    | 940     | ..      |
|  |    | Expected on independent basis         | 805.75               | 780.28 | 108.94 | 852.03  | 77.9573 |
|  |    | Expected on linkage basis—C.O. 29.90% | 886.11               | 712.89 | 105.58 | 919.42  | 3.6912  |
| (ii) Glume (117:139)<br>(one gene common)                              | .. | Observed                              | 940                  | 645    | 290    | 749     | ..      |
|  |    | Expected on independent basis         | 974.40               | 624.60 | 224.86 | 800.14  | 24.0195 |
|  |    | Expected on linkage basis—C.O. 11.48% | 925.24               | 673.77 | 274.01 | 750.98  | 2.4021  |
| Auricle (387:637) with:<br>Glume (117:139)<br>(one gene common)        | .. | Observed                              | 637                  | 373    | 593    | 1021    | ..      |
|  |    | Expected on independent basis         | 594.58               | 397.11 | 594.94 | 1037.37 | 4.7548  |
|  |    | Expected on linkage basis—C.O. 41.14% | 615.57               | 376.11 | 583.68 | 1048.64 | 1.6491  |

PP = Both purple; PW and WP = Recombinant classes; WW = Both white.

Coleoptile segregated in the ratio of 39 purple : 25 white indicating the interaction of three genes—a basic gene ( $A$ ), an inhibitory gene ( $I-Pc$ ) and an anti-inhibitory gene ( $Ai-Pc$ ). Auricle is governed by five pairs of factors—two complementary ( $A$  and  $Pau_a$ ), one inhibitory ( $I-Pau$ ) and two anti-inhibitory-complementary ( $Ai-Pau_a$ ,  $Ai-Pau_b$ ) giving a ratio of 387 : 637. Glume is conditioned by four genes—one basic ( $A$ ), one complementary ( $Pg_a$ ), one inhibitory ( $I-Pg$ ) and one anti-

inhibitory ( $Ai-Pg$ ) suggested by the ratio of 117 : 139.

The ratios of 39 : 25 for coleoptile colour, and 387 : 637 for pigment in auricle are reported for the first time and the ratio of 117 : 139 realised in the case of glume colour is in confirmation with that reported by Ghose *et al.*

The combined segregation presented in Table II has revealed interesting results. These three characters showed the presence of one pleiotropic gene common

to all of them which may be one of the basic-complementary genes designated as *A*. Further analysis indicated the association of these characters. Linkage was detected between the anti-inhibitory genes concerned with them and the cross-over values suggested the sequence of the genes as *Ai-Pg*, *Ai-Pc* and *Ai-Pau* (Fig. 1). Map distances were revised as per the Kosambi formula (Kosambi<sup>3</sup>) and the corrected cross-over value is shown in the parenthesis.

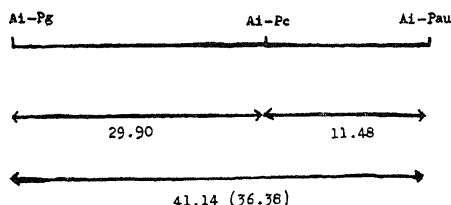


FIG. 1. Linkage map showing relative positions of *Ai-Pg*, *Ai-Pc*, *Ai-Pau* genes. Cross-over values are given in percentages.

Linkage groups in rice have been tentatively constructed by Jodon<sup>2</sup>, Ramiah<sup>7</sup>, Misro *et al.*<sup>4</sup>, and Nagao and Takahashi<sup>5,6</sup> and the linkage group identified and reported for the first time in this paper has not been referred to by them.

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#### NEPHOTETTIX VIRESCENS (DISTANT) NYMPHS AND THEIR ROLE IN THE SPREAD OF RICE TUNGRO VIRUS

RICE tungro virus (RTV) is transmitted by both adults and nymphs of *Nephotettix virescens*<sup>1</sup>. While adults have good mobility, the movement of the nymphs is restricted. Their role in disease

spread is not clearly understood. A replicated experiment was, therefore, set up under field conditions during *Rabi* 1974, using field cages to study the role of nymphs in spread of RTV.

Sixty-three rice plants [Taichung (Native)<sup>1</sup>] with a spacing of 15 × 20 cm were covered by a field cage measuring 1½ × 1½ × 1 meter immediately after transplantation. Twelve such cages were set up. Ten days after transplantation, the middle plant in each cage was replaced by a diseased plant (same cultivar) and caged by cellulose butyrate tubes of 2" diameter. Sixty individuals of each of five instars and adult stages were introduced into each tube separately in a field cage. Leaf hoppers at different stages of development were allowed to feed on the diseased plant for 24 hours to acquire the virus. After acquisition feeding period the cellulose butyrate tubes were lifted and the viruliferous leaf hoppers allowed to migrate on to the healthy plants within the field cage for 24 hours. At the end of this period, the insecticide Carbofuran was used to kill the leaf hoppers. The amount and pattern of infection was recorded 20 days after Carbofuran treatment.

The average percentage of transmission and distance travelled (as indexed by virus infection) by each instar nymph and adult are given in Table I.

TABLE I  
The role of nymphs of *Nephotettix virescens* in spread of rice tungro virus

| Stage of the vector  | Average % infection | Maximum distance travelled in a 85 cm radius within the cage |
|----------------------|---------------------|--|
| First instar nymphs  | 1.6                 | 20   |
| Second instar nymphs | 4.2                 | 35   |
| Third instar nymphs  | 10.1                | 60   |
| Fourth instar nymphs | 18.5                | 75   |
| Fifth instar nymphs  | 20.0                | 75   |
| Adults               | 19.5                | 85   |

The average percentage transmission by first to fifth instar nymphs and adults was 1.6, 4.2, 10.1, 18.3, 20.0 and 19.5, respectively. The later three stages of the leaf hopper could travel for longer distance than the earlier three stages. The third, fourth and fifth instar nymphs and adults were capable of spreading the disease more efficiently than the first and second instar nymphs. The infected plants were located near the inoculum, source in the case of the first, second and third instar nymphs, whereas the fourth and fifth instar nymphs and adults caused infection throughout the field cage though more infected plants were in the vicinity of the inoculum source,

Under natural conditions nymphal populations may be more in number than the adult, especially in the active tillering stage of the crop in the field. It appears, therefore, that nymphs, in all probability, are playing a greater role in disease spread within a field. Presumably, while the adults can bring the primary inoculum to the field, nymphs may aid in secondary spread of the virus. This mechanism may explain the occurrence of the disease in patches (mostly circular) in the initial stages of infection under natural conditions.

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#### SHEATH BLIGHT AND WILT OF RAGI (*ELEusine CORACANA* GAERTN.) CAUSED BY *MARASMIUS CANDIDUS* BOLT.

WILT, disease of ragi (*Eleusine coracana* Gaertn.), a cultivated millet, caused by *Sclerotium rolfsii* Sacc. (Curzi) has been reported to be important in Tamil Nadu and other regions<sup>1</sup>. During the months of June-July 1974, Co. 10, a popular variety of ragi, was found affected to a very serious extent by a sheath blight and wilt in the campus of the Tamil Nadu Agricultural University, Coimbatore. On the sheaths of the affected plants, to a distance of about 5-15 cm from the ground level, characteristic circular to elliptic, necrotic blotches were observed as early symptoms of the disease. As the disease advanced, the sheaths got stuck or bound together, with the mycelium of the fungus, to the stem, eventually leading to wilting and the death of the plants. The diseased plants in a field could be easily distinguished by the discoloured or dried up outer leaves. On lower sheaths of the dead plants small sporophores of a mushroom were noticed. Isolations from the sheaths of the diseased plants yielded a fungus with milky white mycelium. As no sporulation could be obtained, inoculations were made on healthy Co. 10 ragi plants, raised in pots for the purpose, by placing mycelial bits on the sheaths, wrapping them with moist cotton and covering the cotton with a polythene strip to maintain the humidity. Characteristic blight type lesions developed on the inoculated sheaths within a week. Soon the outer leaves turned yellow and dried and the plants eventually succumbed a month after inoculation.

Sporophores of the mushroom developed in 45 to 50 days after inoculation, on the lower ends of the stems thereby proving its pathogenicity.

The fungus is identified as *Marasmius candidus* Bolt. The mushroom is white, delicate, leathery and dry, does not easily decay, but shrivels up in dry weather, and revives in wet weather or when placed in water; pileus membranous, semi-spherical, pellucid, wrinkled; stipe thin; spores hyaline, elliptical to ovate, smooth and measure  $4 \times 2 \mu$ . Basidia four-spored, hyaline; systidia sub-cylindric to ventricose at the basal portion, hyaline and thin; gill trama of loosely interwoven hyphae. These characters agree with other descriptions of *M. candidus* (Bolt)<sup>2,3</sup>.

Quite a few species of *Marasmius* have been known to be pathogenic on cultivated crop plants. *Marasmius sacchari* Wakker, associated with a root necrosis of sugarcane; *M. semiustus* B. et C. (*M. stenophyllus* Mont.) causing death of dryland rice in Malaya<sup>4</sup>, *M. tritici* Yg. on green plants of wheat, rye, barley and quackgrass are a few examples. With the exception of *Pellicularia* (*Sclerotium*) *rolfsii* no other organism has been known to cause wilt of ragi, and this is the first record of sheath blight and wilt caused by *Marasmius candidus* Bolt.

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#### GROWTH INHIBITORS FROM *PARTHENIUM HYSTEROPHORUS* LINN.

PRESENCE of water soluble inhibitor(s) in the fruits and receptacles of *Parthenium hysterophorus* Linn. has been reported earlier from our laboratory<sup>1</sup>. Further experiments were carried out to test if other parts of the weed also contained the inhibitor(s) and to determine the relative concentration in different parts.

The air dried root, stem, leaves, inflorescence and fruits of mature plant of *Parthenium hysterophorus* (0.5 g each) was fragmented and after surface sterilising with 0.1% mercuric chloride and subsequent thorough washing was spread with wheat grains on moist blotters in petridishes at the rate

of six grains per dish and the entire set was replicated five times. Germination and growth of 72 hr old seedlings of wheat were studied. Figure 1 shows that inhibitor(s) is present in all parts of the weed. The concentration of the inhibitor is highest in leaves followed by inflorescence, fruits, roots and stem.

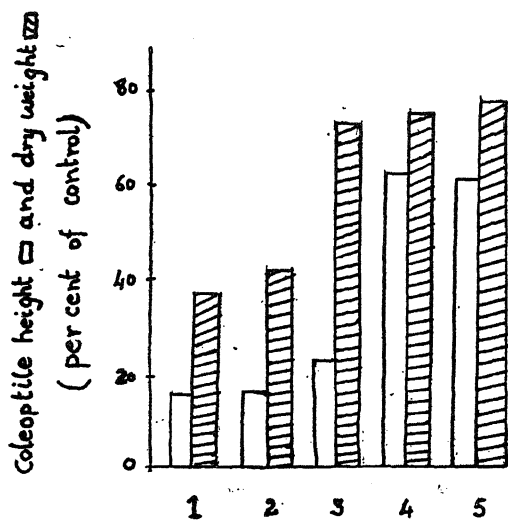


FIG. 1. Parts of the weed tested. 1. Leaves, 2. Inflorescence, 3. Fruits, 4. Roots, 5. Stem.

Further, to analyse the inhibitor complex, to identify the prominent components and to test them for their inhibitory activity, 250 g of air dried stem were cut into small pieces and kept soaked in distilled water for 48 hrs, for the complete leaching out of the inhibitor. Three litres of the brown diffusate were filtered and concentrated to 200 ml. From this the inhibitor(s) was extracted with successive addition of ethyl acetate upto a final volume of 500 ml. The ethyl acetate extract was distilled under reduced pressure at 50–60° C. The thick brownish residue was divided into acidic and non-acidic fractions by extracting the former with 0.1N sodium bicarbonate. This fraction was acidified to pH 2.5 with concentrated HCl and then, the inhibitor was extracted with ethyl acetate (50 ml). This fraction was washed thoroughly with water and concentrated as was also the non-acidic fraction.

The two fractions were run on TLC plates using silica gel and benzene-ethyl acetate 70:30. The spots were developed in an iodine chamber and their  $R_f$  values were calculated. The non-acidic fraction showed 6 spots ( $R_f$  0.88, 0.61, 0.45, 0.28, 0.09). One with the  $R_f$  0.28 was prominent and intense brown. The non-acidic fraction was also chromatographed over acidic alumina column packed with petroleum ether. Petroleum ether

followed by petroleum ether-ethyl acetate 70:30 was run through. The first fraction that was collected crystallised instantaneously and the total yield was 560 mg. The melting point of these crystals was determined and it agreed with that of parthenin (163–166°)<sup>2</sup>. This was further confirmed by chromatographic comparison with an authentic sample of parthenin. This crystalline compound when cochromatographed corresponded to the intense brown spot on the TLC plate referred to earlier. It is interesting to note the presence of parthenin in the aqueous diffusate; it was earlier isolated<sup>2</sup> from petroleum ether and chloroform extracts of the whole plant.

The acidic fraction gave five spots on TLC plate ( $R_f$  0.44, 0.32, 0.20, 0.10, 0.04). The prominent spot with  $R_f$  0.20 was eluted with methanol. Its bright fluorescence under UV light and colour reactions (alcoholic  $\text{FeCl}_3$ -green, bromine water-decolouration,  $\text{AgNO}_3$ -reduction, Gibb's reagent-pink colour) indicated that the compound was a phenolic catechol type, unsaturated acid. Cochromatography with an authentic sample revealed this to be caffeic acid. Using ragi coleoptile bioassay method, the total yield of caffeic acid in the sample was 78 mg.

The spot ( $R_f$  0.32) showing weak fluorescence under UV light, becoming intense on exposure to ammonia and decolourising bromine water, indicated the presence of *p*-coumaric acid. Cochromatography with a pure sample confirmed this.

All the spots in both the acidic and non-acidic fractions were tested for inhibition of growth in the seedlings of ragi (*Eleusine coracana* variety 'Poorna'). All of them except the one with  $R_f$  0.12 in the acidic and  $R_f$  0.46 in the non-acidic fraction answered the test positively.

Thus it may be concluded that all parts of the weed *Parthenium hysterophorus* contain inhibitor(s), its concentration being greatest in the leaves followed by inflorescence, fruits, root and stem. The inhibitor is a complex. Parthenin, caffeic acid and *p*-coumaric acid are the prominent constituents of the inhibitors from the stem.

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### PREVALENCE OF A MOSAIC DISEASE ON FRENCH BEAN IN KUMAON

DURING a survey for virus diseases of cultivated plants in the hilly tracts of Kumaon, Uttar Pradesh, the authors observed French bean (*Phaseolus vulgaris* L.) affected by a mosaic disease in almost all the plots surveyed. The disease incidence varied from 5 to 60%. This caused concern to the growers because French bean is an important vegetable and pulse crop in that area. The diseased plants show varying degree of mottling or chlorosis (Fig. 1), blistering, downward cupping of the lamina. They are rarely killed by the disease but become stunted, dwarfed and bushy depending upon the variety, time of infection and different virus isolates involved. They shed their flowers more freely than the healthy plants and the setting of the pods is adversely affected. This results in loss of yield, in some cases up to 100%.

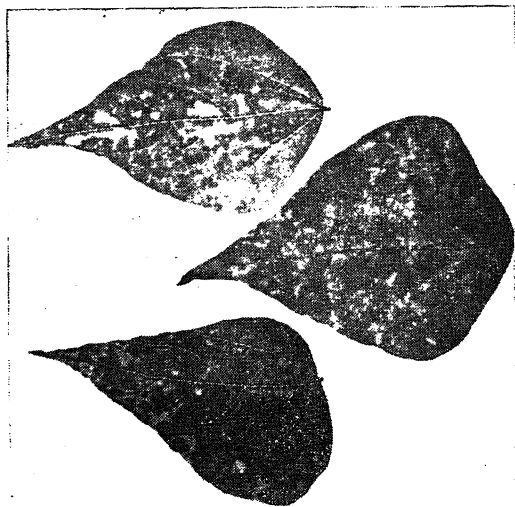


FIG. 1. Infected leaf of French bean.

The disease was mechanically transmitted to a number of bean varieties which include Bountiful, Canadian Wonder, English tender pod, Giant stringless green pod, Masterpiece, Premiar, Prince, Stringless black valentine, Superlative and a few unidentified popular local varieties. All attempts to transmit the disease to the following plants were unsuccessful:

*Brassica oleracea* L., *Capsicum annum* L., *Cucurbita pepo* L., *Cucumis sativus* L., *Datura metel* L., *D. stramonium* L., *Dolichos lablab* L., *Lycopersicon esculentum* Mill., *Nicotiana debneyi* L., *N. glauca* L., *N. tabacum* L. var. *Harrisan special*, *N. rustica* L., *Pisum sativum* L., *Vicia faba* L., *Vigna sinensis* (L.) Endl., and *Zinnia elegans* Jacq.

*Aphis gossypii* Glov. and *Myzus persicae* Sulz. proved to be the most efficient vectors of the virus and transmitted it in a non-persistent manner.

The virus was inactivated at a dilution of 1 : 1000 at temperatures between 55–60° C. Its longevity *in vitro* was 24–32 hours at room temperature (20–22° C). The disease was also transmitted through seed.

Iwanowski<sup>1</sup> was the first to record a mosaic disease of bean from Russia. This was followed by reports of the disease in North America, Australia, South Africa, New Zealand, China, France and Iran. The present study indicates that the virus, responsible for the disease, is a strain of *Phaseolus* virus I similar to that reported by Smith<sup>2</sup>. As regards control, the only method that could be suggested to the farmers is the use of seeds from healthy plants followed by rouging of infected plants. But use of resistant bean varieties would be the best method to get rid of the disease. At present we do not have any resistant variety which could be recommended.

Our sincere thanks are due to Prof. K. S. Bhargava for encouragement and providing the necessary facilities.

Department of Botany,  
University of Gorakhpur,  
Gorakhpur, October 12, 1974.

R. D. JOSHI.  
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A. K. GUPTA.

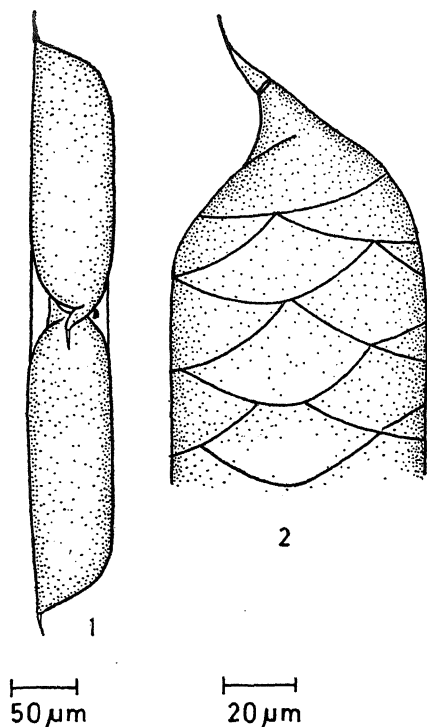
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### OCCURRENCE OF *RHIZOSOLENIA COCHLEA* BRUN (BACILLARIOPHYCEAE) IN PORTO NOVO WATERS

THE present communication deals with the occurrence of *Rhizosolenia cochlea* (Figs. 1, 2) in the neritic waters (Bay of Bengal) of Porto Novo. The species is usually rare in occurrence in coastal areas, although it was reported from Nancowry (Nicobar). (Desikachary—personal communication). The distribution of this species has been discussed recently by Sournia<sup>1</sup> where he mentions about the rarity of this species in the Indian Ocean, reporting its occurrence from restricted regions elsewhere (Siam, Hong-Kong and Japan).

Specimens were isolated from phytoplankton samples collected at the ten fathom lime using a No. 25 bolting silk net on 14th June, 1974. The salinity of the surface water was 35.4‰ and the surface temperature 27.5° C. The technique of

preparing frustules for examination was that given by Hendey<sup>2</sup>.



FIGS. 1-2. Fig. 1. Recently divided cells. Fig. 2. Part of a cell enlarged to show intercalary bands.

There was agreement with the description given by Sournia<sup>1</sup> except in size: the present material measured 58 to 63  $\mu\text{m}$  in diameter (and a range of 50 to 75  $\mu\text{m}$  is also known—Desikachary—personal communication), whereas the range reported by Sournia was 80–130  $\mu\text{m}$ .

The populations of diatoms counted by using the sedimentation technique and an Utermöhl inverted microscope were  $7.4 \times 10^3$  cells/l, of which the population of *R. cochlea* alone was  $4.8 \times 10^2$  cells/l. The associated dominant species were *R. imbricata* Brightwell, *R. calcaravis* Schultze, *Guinardia flaccida* (Castracane) Peragallo, *Bacteriastrium hyalinum* Lauder and *B. delicatulum* Cleve.

Twelve species of *Rhizosolenia* have so far been collected from Porto Novo waters.

The other interesting and rare phytoplankton species observed in these waters were *Chaetoceros glandazii* Mangin (= *C. rostratum*), *C. subtile* Cleve, *Synedra crystallina* (Agardh) Kuetzing and *Campylodiscus echeneis* Ehrenberg among diatoms and *Amphisolenia schroederi* Kofoid and *Oxytoxum elongatum* Wood among dinoflagellates.

The salinity recorded in the present instance (35.4‰) was rather high for Bay of Bengal waters, about the same as the salinity of the Indian Ocean waters. It is known that the South West Monsoon drift (surface current) from April to October flows eastward south of India, with its branches flowing clockwise, following the coastline in the Arabian Sea and the Bay of Bengal<sup>3</sup>. It is possible that *R. cochlea* and some other species could have travelled from the West Indian Ocean (off Madagascar and Africa).

Our grateful thanks are due to Prof. R. Natarajan, Director, for the facilities provided and for constant encouragement. Our profound thanks are also due to Prof. T. V. Desikachary, Centre of Advanced Study in Botany, The University of Madras, and to Dr. A. Sournia of Museum National D' Histoire Naturelle, Paris, for a critical reading of the manuscript and for very helpful comments. One of us (R. S.) is grateful to the University Grants Commission, New Delhi, for the award of a Junior Research Fellowship.

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Tamil Nadu, India, October 11, 1974.

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#### RATIO OF RAY AND FUSIFORM INITIALS IN THE VASCULAR CAMBIUM OF SOME ARID-ZONE PLANTS

As far back as 1923, Bailey<sup>1</sup> had noted that the fusiform cells occupy approximately seven-eighth of the total circumference of the cambial zone in *Pinus*, a much lesser proportion in many dicotyledons and a little less than one half in certain extreme cases. But recently Wilson<sup>6</sup> calculated the surface area of the different cambial initials in *Abies concolor* and found that the fusiform cells form more than 90% by volume of the cambium and its derivatives. Based on the above, Wilson<sup>7</sup>, in the year 1964, proposed a model for the cambium of conifers. Following this, Kozłowski<sup>5</sup> (p. 7) also noted a similar composition for the cambium in general in his book on "Growth and development of trees". Butterfield's<sup>2</sup> recent observations on



*Aeschynomene hispida* also made it clear that the fusiform cells may form more than 95% of the cambial population in certain cases.

Contrary to the above, Ghouse and Yunus<sup>4</sup> observed that the fusiform cells do not constitute more than 60% of the cambium in the fully grown trees of *Dalbergia*, although their magnitude may go up to 80% in the young trees. Again these authors found the fusiform cells forming only 25% of the cambial population in one of the tropical trees, viz., *Dillenia indica* (in Press).

Keeping in view the above developments, the present work was undertaken with an aim to find out in what proportion the fusiform and ray initials occur in the cambial zone of certain arid-zone plants.

Cambial samples, along with some conducting phloem, and sapwood collected (20 samples per species) from the main trunks of *Acacia catechu* Willd., *A. farnesiana* Willd., *A. melanoxylon* R.Br., *A. nilotica* (Linn.) Willd. var. *kauria* or *vedi*, *A. nilotica* var. *Ramkanta* or *Ramkati*, *A. nilotica* var. *telia* or *godi*, and *Prosopis spicigera* Linn., fixed in F.A.A., preserved in 70% ethanol were sectioned on a sliding microtome in tangential plane at a thickness of 10–12  $\mu$ , stained with tannic acid-ferric chloride<sup>3</sup>, mounted in Canada balsam after dehydration in ethanol series and studied. Camera lucida diagrams were made on tracing paper, out of all samples and the portions containing ray initials were removed and weighed. The portions containing fusiform initials (after removing ray initials) were then weighed separately. The weighing was done on a sensitive chemical balance. The proportion of fusiform initials to ray initials per unit area was calculated on the basis of the weights thus obtained.

Analysis of the data obtained in the present study revealed, that the fusiform cells constitute at their maximum about 82% of the total tangential area of cambial zone in *A. melanoxylon*. This is closely followed by *A. farnesiana* and *A. nilotica* var. *Ramkanta*, in which the fusiform initials occupy 78% and 75% of the total tangential area respectively. In *A. catechu* fusiform cells occupy about 70% while in the other two varieties of *A. nilotica*, viz., *telia* and *kauria*, the fusiform cells form about 62% and 57% respectively. In case of *Prosopis* the fusiform cells take about 63% of the total tangential area, while the remaining 37% of the cambial zone is occupied by the ray initials (Fig. 1).

The results, thus, clearly indicate that in no species, investigated the proportion of fusiform cells exceeds more than 82% (57–82% in *Acacia* and 63% in *Prosopis*), and assume a magnitude of

90% or more, as it was reported by some earlier workers<sup>2,5-7</sup>.

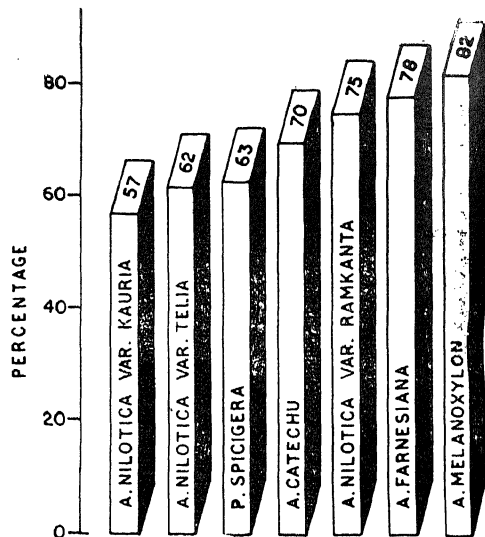


Fig. 1

The second author (M. Y.) is very thankful to the Council of Scientific and Industrial Research, New Delhi, for the award of a Senior Fellowship. The laboratory facilities provided by Prof. R. Khan, are being gratefully acknowledged by all.

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October 15, 1974.

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MOHD. YUNUS.  
MOHD. IQBAL.

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#### A NEW GRASS FROM NEPAL

Borl<sup>1</sup> has mentioned 18 taxa of genus *Eulalia* Kunth (Poaceae). One more new taxon has been found from Nepal. This new taxon is named and described here as *Eulalia trispicata* (Schult.) *Henr. var. hookeri* P. Sur var. nov.

*Eulalia trispicata* (Schult.) *Henr. var. hookeri* P. Sur, var. nov.

Differt a varietate typica habitu parbiore, Spiculis longioribus, lemmate superiore bilobo, apicibus acuminatis.

This variety differs from *Eulalia trispicata* (Schult.) Henr. mainly in its habit, longer spikelets and bilobed upper lemma with acuminate tips.

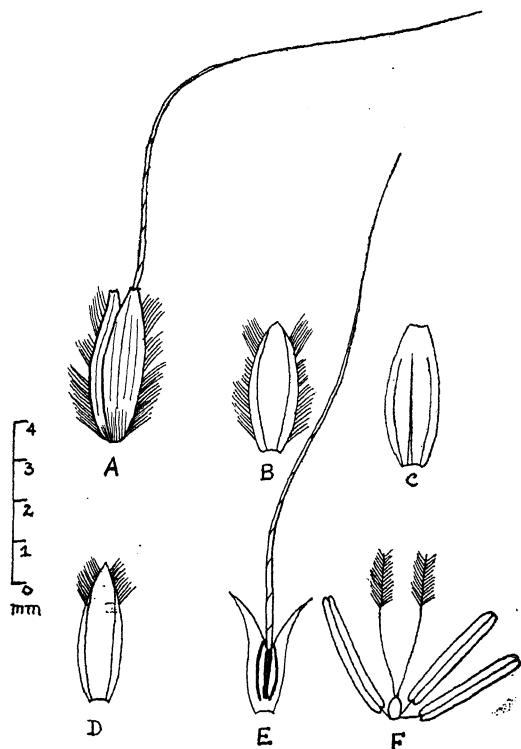


FIG. 1. *Eulalia trispicata* (Schult.) Henr. var. *hookeri* P. Sur var. nov. A, Sessile spikelet; B, Lower glume; C, Upper glume; D, Lower lemma; E, Upper lemma; F, Stamens and pistil.

*Culms* 25 cm long, slender, branched. *Leaves* 3–15 cm long and 1–2 mm broad, narrowly linear, glabrous; *ligules* short, membranous, fringed with long hairs. *Racemes* 4–6, narrow, 3–5 cm long, compressed; pedicels of spikelets flattened. *Sessile spikelets* 3–3.5 mm long. *Lower glume* 3 mm long, membranous above with a hyaline tip; margins narrowly incurved, villous with long silky hairs. *Upper glume* 3.5 mm long, oblong; margins 1-nerved incurved. *Lower lemma*—empty, 3.5 mm long, narrowly oblong, hyaline, flat, nerveless. *Upper lemma* 3 mm long, linear-oblong, divided into two lobes; lips of lobes acuminate; awns slender, 12–13 mm long, *palea* absent. *Anthers*—3, 3 mm long. *Pedicelled spikelets* similar to the sessile.

*Holotype*: Nepal, Kukhure Dol (Phul), Alt. 7000 ft, 5 October 1967, Mrs. Prodhan 7472 (CAL).

*Etymology*: The grass is being named in honour of the well-known botanist Sir J. D. Hooker.

I am grateful to Deputy Director and Keeper, Central National Herbarium, Howrah, for their encouragement, Dr. R. B. Mazumder, Systematic Botanist, for valuable suggestions and Dr. N. C. Mazumder, for Latin diagnosis.

Central National Herbarium,  
Botanical Survey of India,  
Botanic Garden,  
Howrah-3, October 14, 1974.

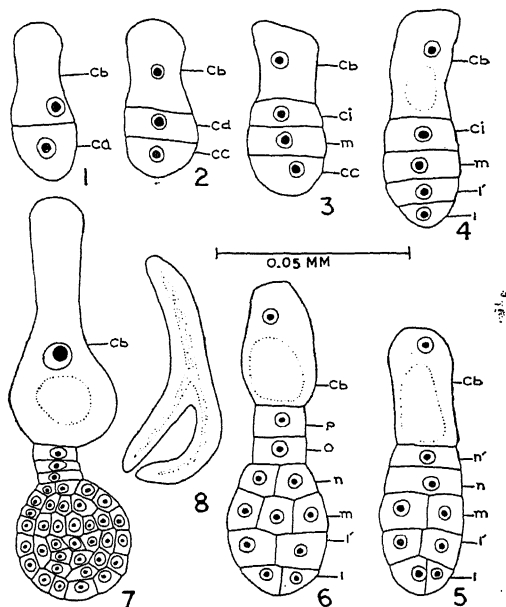
P. R. SUR.

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### DEVELOPMENT OF EMBRYO IN *STELLARIA GLAUCA* WITHERING (CARYOPHYLLACEAE)

THE embryology has been a fascinating subject to many embryologists. Very few contributions have appeared so far on the development of embryo of the members of Caryophyllaceae family<sup>1</sup>. Recently, Pal and Murty<sup>2</sup> have described the embryo development in *Stellaria aquatica*. The present note gives some information regarding the development of embryo in *S. glauca*.

The zygote first divides by a transverse division forming a terminal cell *ca* and a basal cell *cb* (Fig. 1). The basal cell *cb* remains undivided



FIGS. 1–8. Figs. 1–6. Stages in the development of embryo. Fig. 7. A globular embryo with a short suspensor and vesicle like basal cell. Fig. 8. Mature embryo showing procambial strand.

the embryo now takes further part in the development of primary and tertiary large vascular structures (Figs. 2-7). The terminal cell in undergoes transverse division and thus two cells are formed, an upper and a lower cell (Fig. 2). The cell of upper undergoes a transverse division and gives rise to *Q* and *P* (Fig. 3). The cell of lower divides transversely, forming *Q* and *P* (Fig. 4). Thus a row of four cells is formed from *Q* and *P*. Of these the inner cells (*Q* and *P*) divide by a vertical division and the outer cells (*Q* and *P*) shows a transverse wall (Fig. 5). The embryo now shows the formation of *Q* and *P*. In *Q*, a vertical wall is also developed (Fig. 6). In *P*, a transverse wall is formed (*Q* and *P*) (Fig. 7). In this way six tiers of cells are formed. The tier *Q* gives rise to stem tip, the completion of the culm up to root cap and *O*. *P* is short suspension.

A further embryo (Fig. 8) is formed with a short suspension and a very low basal cell, as a result of further divisions of *Q* and *P*. In the usual way, stem tip, cotyledons, etc., get differentiated (Fig. 8). When differentiation is complete there is formation of proembrya strands (Fig. 8). Thus the embryo development conforms to *Carpophyllid* type, as has been reported for *Salicornia*, *Apetalia*, *Stenandria* and *S. aquatica*.

The author is greatly indebted to Prof. Y. S. Murty for encouragement and Dr. V. Singh for providing the facilities and for his keen interest.

School of Plant Morphology, S. P. T. Meerut College,

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### CULMS IN CYPERUS

IN an attempt to the anatomy of Cyperaceae, Metcalfe (1971) has stated that culm anatomy of *Cyperus esculentus* somewhat resembles *Cyperus articulatus*. David-Jouve (1974) examined the culm anatomy of *Cyperus aureus* which he considered identical with *C. esculentus*. Anderson (1888), Plowman (1906), Buchholz (1921), Greiss (1957), Morita (1963), Shyam (1963), Shah (1967), Mehra and Sharma (1970) and Fisher (1970) have described the stem anatomy of many species of *Cyperus*, but *C. esculentus* is least mentioned. D'Almeida and Ramaswamy (1948) studied the anatomy of 10 Indian species of *Cyperus*,

but *C. esculentus* is not worked out. Thus, the literature shows that the details of the culm anatomy of this species have not received due attention by anatomists.

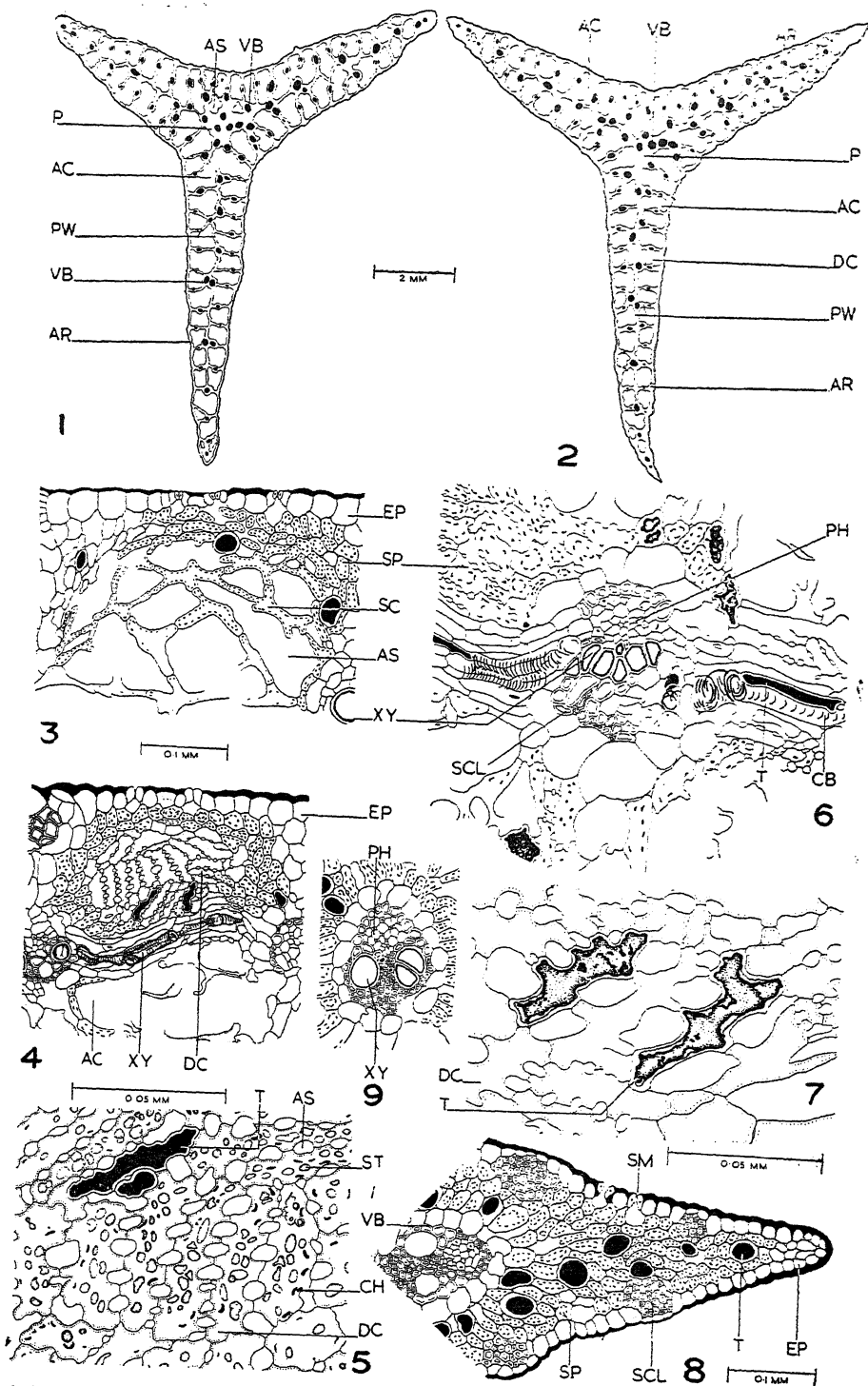
The inflorescence axis or the culm of *C. esculentus* is easily distinguished by its considerable length and triangular outline. It is straight and unbranched, and arises singly. The maximum length of the culm recorded in the month of August was 176 cm. Underground part of the culm is delicate and white, while the aerial part assumes a light green colour.

Culm is Y-shaped in a cross-section (Fig. 1). Many continuous air canals, separated by thin, delicate partition walls of parenchymatous cells (Figs. 1 and 2), are present all along its length. Partition walls are only one cell thick (Fig. 3). The many vascular bundles lie at intersections of these partition walls. These air canals are divided into a number of small compartments by horizontal partition walls forming diaphragms (Fig. 4). Diaphragms have also been recently reported in the leaves of this species by Shyam and Sharma (1974). These diaphragms are always supported by a cross-bundle which connects the two main bundles, running longitudinally throughout the body of the culm (Figs. 4 and 6).

The inner borders of these air canals are lined by parenchymatous cells containing abundant chloroplasts. But in the centre of the compartment, there are many small intercellular spaces, which give the walls, a beaded appearance in transverse section (Fig. 3). Diaphragm cells vary from polygonal to stellate in shape. These are present in groups, and are very long, but narrow, with short arms (Fig. 4). More cytoplasm is present in the diaphragm cells in comparison to that of the other cells (Fig. 5). They also contain some starch grains, and some of the diaphragm cells are completely filled with tannin (Fig. 7). The cross-bundles are made up of xylem and phloem, which are connected with the xylem and phloem of the longitudinal bundles of the culm (Fig. 4). Sometimes, a single longitudinal bundle is seen, connected with the two cross-bundles on either side (Fig. 6).

The longitudinal bundles of the culm are exclusively collateral, and have fiber cells on their outer side. The bundles are arranged in two sets, the outer alternating with the large air canals, and are arranged along the entire length of the arm of culm in two rows, while the inner are irregularly scattered in the culm (Fig. 2).

However, at the three angles of the culm, the margins are extremely pointed (Fig. 2). They are interrupted by two to three vascular bundles in a single row. The xylem and phloem are completely



Figs. 1-9. Figs. 1-2. Serial transverse sections of culm passing from base upwards at different levels. Fig. 3. T.S. of culm showing the stellate cells in the air canal. Fig. 4. T.S. of a portion of culm showing diaphragm, supported by a cross-bundle and oblique bundles. Fig. 5. T.S. of a portion of culm showing the stellate cells in the air canal. Fig. 6. T.S. of a portion of culm showing the stellate cells in the air canal. Fig. 7. T.S. of a portion of culm showing the stellate cells in the air canal. Fig. 8. T.S. of a portion of culm showing the stellate cells in the air canal. Fig. 9. T.S. of a portion of culm showing the stellate cells in the air canal.

Fig. 5. T.S. of a portion of diaphragm showing contents. Fig. 6. T.S. of a portion of culm showing a longitudinal vascular bundle connection on either side by two cross-bundles. Fig. 7. T.S. of a portion of culm showing two tannin-filled cells of diaphragm. Fig. 8. T.S. of one of the three angles of a culm showing a collateral vascular bundle, spongy parenchyma, hypodermal ribs, and a few tannin-filled cells. Fig. 9. T.S. of a vascular bundle.

(AC, air canal; AR, arm of culm; AS, air space; CB, cross-bundle; CH, chloroplasts; DC, diaphragm cells; EP, epidermis; P, parenchyma; PH, phloem; PW, partition wall; SC, stellate cell; SCL, sclerenchyma; SM, stoma; SP, spongy parenchyma; ST, starch grain; T, tannin; VB, vascular bundle; XY, xylem.)

separated in the mature bundles by a plate of sclerotic tissue (Fig. 9). Chlorophyllous parenchyma forms a compact layer underlying the epidermis. The mechanical tissue of the culm is confined to a few, very small, hypodermal ribs, and a few sclerenchymatous strands above the partition walls, separating the air canals and surrounding the vascular bundles. These hypodermal ribs are more prominent in the angles of the culm (Fig. 8), and are composed of small sclerenchymatous cells. All these structures are surrounded by epidermis, which is covered by a thin cuticular layer. The cuticle, though thin, is conspicuous at the extreme edges of the culm (Fig. 8). Over the hypodermal ribs, the epidermal cells are small. Two or three stomata, which are somewhat depressed, open into the air canals.

School of Plant Morphology,

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Meerut (India), October 1, 1974.

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## SHORT SCIENTIFIC NOTES

### A New Leaf Spot Disease of *Ipomoea carnea* Jacq.

A leaf spot disease of *Ipomoea carnea* Jacq. was observed during the summer season around Madanapalle. The disease is characterised by large irregular dark brown necrotic areas, often lightly flecked with dark conidia. In later stages the central portions fall off giving a 'shot-hole'-like appearance.

The fungus was isolated and pathogenicity proved by spraying the spore suspension on detached twigs. The spores (conidia) were pale brown, muriform, obclavate with long beaks. These conidia with cross (2-7), longitudinal (1-8) and oblique (1-4) septa measure  $36$  to  $75\mu$  (with beak) in length and  $6$  to  $12\mu$  in diameter. The length of the beak varies from  $6$  to  $45\mu$ . The confirmation of the causal organism as *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire by Dr. Ellis of CMI, Ferry Lane, Kew, England (IMI 184577) is gratefully acknowledged.

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serious pathogen causing foot and crown rot of wheat in other countries.

The author is thankful to Director, C.M.I. Kew, Surrey, England, for the identification of the fungus.

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Palampur (H.P.), November 15, 1974.

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### Evidence of Polyploidy in *Stephensiella brevipedunculata* Kash.

*S. brevipedunculata* has been cytologically investigated by Mehra<sup>2</sup>, who has reported eight mitotic chromosomes from gametophytic tissue. He did not come across any meiotic abnormality. The present author has also confirmed eight small ( $1.5\mu$ - $2\mu$  long) chromosomes in the androgonial cells, but the findings of meiotic configurations clearly rule out the possibility of this species from Naini Tal to be at the monoploid level.

The thalli of *S. brevipedunculata* grow luxuriantly in different places of Kumaon and the material for the present study was collected from Naini Tal. Young antheridia and sporogonia were collected and fixed in July and August 1973. The method adopted for fixing, etc., was as in the previous case<sup>2</sup>.

On examination of thirty-three dividing spore mother cells, the following groupings of the sixteen chromosomes were observed.

A. In 25% cells, chromosomes were arranged in four groups: each one being a tetravalent.

B. In 60% cells, six groups were observed consisting of two tetravalents and four bivalents.

C. In 9% cells, eight groups were seen, out of which one was tetravalent, five bivalents and two univalents.

D. In 6% cells eight groups of bivalents were observed.

These findings clearly indicate that *S. brevipedunculata* from Naini Tal is at the diploid level with  $2n=8$  and it might have evolved from some haploid member of the Hepaticae with  $n=4$ . This view is in concurrence with the opinion of Berrie<sup>1</sup> and Proskauer<sup>3</sup>, according to whom all the ancestors

### Foot Rot of Wheat Caused by *Fusarium graminearum* Schwabe.

The nature of foot rot and seedling blight of wheat is not fully known in India. Eleven fungi have been reported to cause root rot and foot rot<sup>1-3</sup>. Wheat plants grown at the farm, College of Agriculture, Palampur (H.P.), showed necrosis of culm at soil level. The culm gets blackened and the plants are stunted in growth. Some of the plants are broken from that portion and are lodged whereas the other (in which blackening is less) stand normally. All such plants have smaller ears compared to healthy plants. *Fusarium graminearum* was isolated from such plants from culm portion. The pathogenicity of the fungus was proved by adding the culture to the soil. The seeds were sown afterwards. Only 38% seedlings survived and produced healthy ears. 20% plants died at seedling stage and 42% at maturity time. These plants showed typical foot rot symptoms. This study revealed that *Fusarium graminearum* Schwabe causes foot rot of wheat. *F. graminearum*, though not reported from India, has been known to be a

of *Hepaticae* had evolved from *Takaka* ( $n=4$ ) through doubling of chromosomes.

Mehra's failure to observe evidence of diploidy in species growing in these areas (W. H. and Punjab plains) may have been due to 100% incomplete chromosomal association at pachytene (6% in present findings). He has however reported diploidy in *Asterella reticulata*, *A. blumeana* and *Athalia pinguish*.

I am thankful to Dr. Ram Udar for his guidance during the preparation of this manuscript.

Department of Botany, H. S. KANWAL.  
Kumaon University,  
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1. Berrie, G. K., *Trans. Brit. bryol. Soc.*, 1958 b, 3, 427.
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***Polistes hebraeus* (Fabricius) Preying upon *Rhipiphoro-  
thrips cruentatus* Hood (Thripidae:Thysanoptera)**

*Rhipiphorothrips cruentatus* Hood is a polyphagous species and has been reported to feed upon *Vitis* spp., *Lagerstroemia indica*; *Punica granatum* Linn.; *Syzygium jambolana* (Linn.); *Careya arborea* Roxb.; *Anacardium occidentale* Linn.; *Terminalia catappa*, *Mangifera indica* and *Rosa* sp.<sup>1,2</sup>. During survey on pests of ornamental trees and shrubs at Ludhiana in the months of August–November, 1974, rose plants were found to be attracting large number of the workers of the yellow wasp, *Polistes hebraeus* (Fabricius). Observations were, therefore, made on the status of this wasp in the rose ecosystem.

On close observation, the adult wasps were found feeding on the nymphs of the thrips. The workers started their activity at 09 hr and continued till 17 hr daily. On an average, a single shoot of the rose infested by the thrip was visited 14 times per hour and 3–5 minutes were spent per shoot by the nymph searching wasp which located its prey in the young tender shoots with the help of to and fro movements of its antennae. The starved wasps when put in glass jars (10 × 15 cm) along with the rose shoots each having twenty nymphs in each jar, it devoured 12–16 nymphs in an hour which further confirmed its predatory role.

Ananthakrishnan<sup>1,2</sup> mentioned several insect-enemies of thrips but it seems to be a first record of *Polistes hebraeus* (Fabricius) being predaceous on *Rhipiphorothrips cruentatus* Hood feeding on roses.

The author is highly thankful to Dr. T. N. Ananthakrishnan, Entomology Research Unit, Loyola College, Madras-34, for the identification of the thrips.

College of Agriculture, J. S. DHALIWAL.  
Punjab Agricultural University,  
Ludhiana, December 17, 1974.

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***Sophora tomentosa* Linn.—A New Host for *Macrosiphum* (*Acyrtosiphon*) *gossypii* Mordv. (Homoptera: Aphididae)**

The aphid, *Macrosiphum* (*Acyrtosiphon*) *gossypii* Mordv. has been recorded on cotton and described its pest<sup>1</sup>. Two subspecies of this insect, *pactoskii* and *turanicum* were observed on stems of *Lepidium perfoliatum*<sup>1</sup> and cotton<sup>2</sup> respectively.

During March 1974, *Sophora tomentosa* Linn. a leguminous evergreen shrub cultivated in gardens around bungalows was found very severely infested with *M. (A.) gossypii* Mordv. in the nursery at Haryana Agricultural University, Hissar, India. Due to damage done by this aphid the growth of the plant was very much retarded. So far cotton and *Lepidium perfoliatum* are the only recorded hosts of this aphid. Hence, *S. tomentosa* Linn. is a new host for *M. (A.) gossypii* Mordv.

Sincere thanks are due to Dr. T. P. Yadava for providing facilities and to Dr. S. Kanakaraj David for identifying the insect.

Department of Plant Breeding, N. D. VERMA.  
Oilseeds Section (Entomology), H. V. SINGH.  
Haryana Agricultural University,  
Hissar (Haryana), March 20, 1975.

1. Mordvilko, A., *Memoirs of the Bureau of Entomology of the Scientific Committee of Central Board of Land Administration and Agriculture*, Petrograd, 1915, 8 (3), 54.
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## REVIEWS AND NOTICES OF BOOKS

### Absorption Spectroscopy of Organic Molecules.

By V. M. Parikh. (Addison-Wesley Pub. Co., Reading, Massachusetts 01867, U.S.A.), 1974. Pp. x + 325. Price not given.

The book is intended mainly to undergraduate students of different disciplines who want to get initiated into spectroscopy. Accordingly, the author begins with a quick review of basic physics in Chapter 1. The book covers in Chapters 2, 3, 4 and 5 in considerable detail the fields of uv, ir, n.m.r. and mass spectrometry. Basic principles of each technique instrumentation, interpretations of spectra and applications are discussed in each chapter. Chapter 6 is devoted to the integration of various techniques. The author has given many spectroscopic problems. Researchers and practising chemists may find the book useful as a source of quick reference (covered upto 1971 only). The author has included extensive charts of spectroscopic correlation in the appendix, again useful to practising chemists.

The get-up of the book is excellent and appealing, and it is free from typographical errors.

T. R. KASTURI.

### Chemistry : A Unified Approach (3rd Edition).

J. W. Buttle, D. T. Daniels and P. J. Beckett. (Butterworths, London, WC. 2 B4 AB), 1974. Pp. x + 580. Price £2.95.

The book as the authors have pointed out may be divided broadly into three parts. The first part from chapters one to seven deals with the Physical aspects of chemistry such as the development of the structure of the atom, the concept of atomic orbitals, nature of the chemical bond, properties of the different states of matter, properties of solutions, thermochemical concepts, reaction rates, chemical equilibria, redox potentials and other related concepts relevant to the understanding of the behaviour of chemical elements and their compounds.

The second part from chapters eight to seventeen discusses the origin and distribution of the elements in the earth, their extraction from the ores, the properties and commercial applications of these elements and their compounds. That there is a theoretical basis for the properties exhibited by the elements as well as their compounds has been brought out throughout the discussion in an easily

understandable way. Special emphasis has rightly been given to the structural features of the elements and their compounds.

Chapters eighteen to twenty-six deal with the chemistry of organic compounds—their sources, reactions and applications. Throughout this discussion wherever possible an attempt has been made to educate the student on the probable reaction mechanism.

In addition to these three main divisions there is a brief but useful discussion of the modern experimental methods in chapter twenty-seven and an excellent introduction to "the aspects of chemical industry" in chapter twenty-eight. At the end of each chapter a number of questions are given which the student may profitably attempt to solve and thus enhance his understanding of the subject. Answers to several numerical problems are also given at the end of the book. Throughout the book the subject-matter has been kept lively and interesting and the approach has been completely modern.

The very fact that the book has gone into its third edition within a short period of eight years is a measure of its success. The authors have intended the book for students taking the pre-degree and general degree courses in Great Britain. It could certainly be recommended for students taking the degree courses of Indian Universities. Though it may not give all the information that an undergraduate of an Indian University requires to face his examination, it certainly excels other books of comparable standard in several respects and has information and ideas that every undergraduate student of chemistry ought to know.

G. K. NARAYANA REDDY.

### Books Received

*Solid State Chemistry*. Edited by C. N. R. Rao. (Marcel Dekker, Inc., 305 E. 45th St., New York, N.Y. 10017), 1974. Pp. x + 918. Price \$59.50.

*Advanced Soil Physics*. Edited by C. Dakshinamurti. (Indian Council of Agricultural Research, New Delhi-1), 1972. Pp. x + 249. Price Rs. 15.00.

*The Grass Cover of India*. By P. M. Dabadchao and K. A. Sankaranarayan. (Indian Council of Agricultural Research, New Delhi-1), 1973. Pp. xii + 712. Price Rs. 35.00.



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# MANGANOAN CUMMINGTONITE FROM SAKARSANAHALLI, KOLAR DISTRICT

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## ABSTRACT

Manganoan cummingtonite from the carbonate horizons of Sakarsanahalli is unique in containing  $2.45 \text{ Mn}^{2+}$  per formula unit. The manganese content of this mineral is the highest that has been reported so far. It has a  $\text{Mg}:\text{Mn}:\text{Fe}$  ratio of  $49:42:9$ . The unit cell dimensions are  $a = 9.598 \text{ \AA}$ ,  $b = 18.072 \text{ \AA}$ ,  $c = 5.266 \text{ \AA}$ ,  $\beta = 103^\circ 44'$ . The  $\beta$ -angle corresponds to a value between that of tremolite and cummingtonite, with a smaller  $c$ -value. This can only be attributed to the enrichment of  $\text{Mn}^{2+}$  in the  $\text{M}_4$  site with a possible 8-coordination as against the normal 6-coordination.

It is generally believed that manganese content in the monoclinic iron-magnesium amphiboles  $(\text{Mg}, \text{Fe})_7\text{Si}_8\text{O}_{22}(\text{OH})_2$  (cummingtonite-grunerite series) will not exceed 2 atoms per formula. This originates from the expectation that the larger divalent cations preferentially occupy the  $\text{M}_4$  sites in the crystal structure, whereas the smaller cations fill in the various  $\text{Y}(\text{M}_1, \text{M}_2, \text{M}_3)$  positions, all the sites having distorted octahedral coordination<sup>1</sup>. Contrary to this view, a unique amphibole containing  $2.45$  atoms per formula ( $20.0\%$  by wt.  $\text{MnO}$ ) has been collected from Sakarsanahalli, Kolar District (Lat.  $12^\circ 48'$ ; Long.  $78^\circ 13'$ ). The amphibole occurs in the metamorphosed "silicate-carbonate" horizon of an abandoned manganiferous limestone quarry, west of the village. The quarry is a part of the complex rock exposures occurring as isolated small rises within the granites and gneisses of the south-west margin of Kolar Schist belt. The chief rock types of the complex are: carbonates ( $\text{Mn}$  — calcite +  $\text{Mn}$  — dolomite + amphiboles), ferruginous schists (quartz + haematite + magnetite + anthophyllite + talc), metapelites (cordierite + sillimanite + muscovite + quartz), pyroxenites (diopside + garnet + calcic amphiboles + clinozoisite + quartz) and amphibolites (hornblende + plagioclase + quartz). Manganese is a common substituent to varying degree in pyroxenes, garnets, and amphiboles. Tremolite and cummingtonite are the normal constituents of the carbonate rock.

Manganoan cummingtonite occurs as tufted acicular fibres along the localised shear planes in the carbonate rocks. The fibres could easily be handpicked and the sample thus obtained is treated with dilute hydrochloric acid. Microscopic examination reveals that the phase purity is over  $99\%$ . The amphibole is pale glistening white to light brown in colour. Most of the crystals are in the form of fine fibres (asbestiform). However, long slender transparent crystals can often be seen which show acicular and prismatic habits (Fig. 1). They

are non-pleochroic and show lamellar twinning parallel to  $c$ -axis. The extinction angle varies from  $15^\circ$  to  $18^\circ$ . The biaxial needles are optically negative. The optical properties are given in Table I, which corresponds to the manganoan cummingtonite reported by Jaffe *et al.*<sup>2</sup>. Except by chemical means, it is often difficult to conclusively identify this mineral. It may be mistaken for tremolite asbestos which has optical properties similar to manganoan cummingtonite.

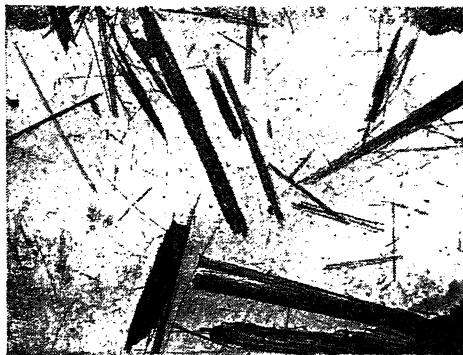


FIG. 1. Manganoan cummingtonite, Sakarsanahalli, Magnification,  $\times 25$ .

## CHEMISTRY

The carbonates, the cummingtonite and the coexisting tremolites are analysed by wet chemical methods. Cations are estimated complexometrically; water and carbon dioxide by gas effluent analysis. No fluorine is detected in the sample (Tables I and II). The analytical data show that the amphibole under consideration is manganoan cummingtonite with the formula  $(\text{Mg}_{2.9}\text{Mn}_{2.5}\text{Fe}^{2+}_{0.8}\text{Ca}_{0.3}\text{Al}_{0.3})\text{Si}_{7.99}\text{O}_{21.8}(\text{OH})_{2.1}$ . According to Jaffe *et al.*<sup>2</sup>, the monoclinic  $\text{Mg}$ – $\text{Mn}$ – $\text{Fe}$  amphibole containing 50 or above mole per cent  $\text{Mg}$  is to be called cummingtonite even though the manganese substituted mineral is optically negative. The  $\text{Mg}:\text{Mn}:\text{Fe}^{2+}$  is  $49:42:9$  for the present cum-

TABLE I  
Chemical composition of amphiboles

|  | Manganooan<br>Cumming-<br>tonite | Manganooan<br>Actinolite I | Manganooan<br>Actinolite II |
|--|----------------------------------|----------------------------|-----------------------------|
| SiO <sub>2</sub>                               | 55.14                            | 50.11                      | 54.74                       |
| TiO <sub>2</sub>                               | 0.00                             | 0.28                       | 0.49                        |
| Al <sub>2</sub> O <sub>3</sub>                 | 2.85                             | 1.24                       | 3.57                        |
| Fe <sub>2</sub> O <sub>3</sub>                 | 0.08                             | 1.71                       | 0.94                        |
| FeO  | 4.52                             | 13.82                      | 7.48                        |
| MnO  | 20.03                            | 6.30                       | 9.61                        |
| MgO  | 13.25                            | 11.67                      | 9.40                        |
| CaO  | 2.02                             | 12.32                      | 10.62                       |
| Na <sub>2</sub> O                              | 0.21                             | 0.56                       | 0.87                        |
| K <sub>2</sub> O                               | 0.05                             | 0.07                       | 0.20                        |
| H <sub>2</sub> O <sup>-</sup>                  | 2.17                             | 1.83                       | 1.98                        |
| H <sub>2</sub> O <sup>+</sup>                  | 0.04                             | 0.06                       | 0.15                        |
| Total  | 100.27                           | 99.97                      | 100.05                      |
| $\alpha$                                       | 1.629                            | 1.647                      | 1.638                       |
| $\beta$  | 1.649                            | 1.663                      | 1.652                       |
| $\gamma$                                       | 1.652                            | 1.669                      | 1.661                       |
| 2V   | 75°                              | 74°                        | 84°                         |
| Z <sub>Ac</sub>                                | 17°                              | 16°                        | 20°                         |
| D  | 3.26                             | 3.19                       | 3.17                        |
| Number of cations on the basis of 24 (0) atoms |                                  |                            |                             |
| Si   | 7.989                            | 7.565                      | 7.966                       |
| Al   | 0.011                            | 0.221                      | 0.034                       |
| Al   | 0.476                            | ..                         | 0.578                       |
| Ti   | 0.000                            | 0.032                      | 0.054                       |
| Fe <sup>+3</sup>                               | 0.009                            | 0.194                      | 0.103                       |
| Fe <sup>+2</sup>                               | 0.549                            | 1.745                      | 0.930                       |
| Mn   | 2.462                            | 0.806                      | 1.156                       |
| Mg   | 2.867                            | 2.626                      | 2.039                       |
| Ca   | 0.315                            | 1.996                      | 1.658                       |
| Na   | 0.059                            | 0.164                      | 0.245                       |
| K  | 0.011                            | 0.014                      | 0.037                       |
| (OH)   | 2.103                            | 1.845                      | 1.924                       |

TABLE II  
Chemical composition of carbonates

|                               | Kutno-<br>horite<br>(1) | Manganooan<br>Calcite<br>(2) | No. of cations:<br>Basis 6 (0) atoms<br>(1) | (2)   |
|-------------------------------|-------------------------|------------------------------|---|-------|
| FeO                           | 0.28                    | 1.55                         | Fe 0.008                                    | 0.044 |
| MnO                           | 24.30                   | 14.40                        | Mn 0.715                                    | 0.411 |
| MgO                           | 4.12                    | 5.80                         | Mg 0.213                                    | 0.291 |
| CaO                           | 29.35                   | 34.72                        | Ca 1.093                                    | 1.253 |
| CO <sub>2</sub>               | 41.90                   | 43.55                        | C 1.986                                     | 2.001 |
| H <sub>2</sub> O <sup>+</sup> | 0.13                    | 0.11                         |   |       |
|                               | 100.08                  | 100.13                       |   |       |
| $\epsilon$                    | 1.525                   | 1.495                        | $a, \text{\AA}$ 4.84                        | 4.89  |
| $w$                           | 1.713                   | 1.683                        | $c, \text{\AA}$ 16.27                       | 16.58 |
| D                             | 3.13                    | 2.92                         |   |       |

ingtonite. Manganese substituted cummingtonite-grunerites are known by names like tirodite<sup>3,4</sup> (5 to 10 wt% MnO and minimum FeO) and danne-morite<sup>5,6</sup> (7.4% MnO and 24 wt% FeO). Jaffe *et al.*<sup>2</sup> have reported a manganooan cummingtonite with 2.28 Mn<sup>2+</sup> per formula unit. This analysis is doubted because of the spessartite inclusion in the sample and that the analysis is carried out spectrographically. Klein<sup>7</sup> has analysed three cummingtonites containing 2.02, 1.74 and 1.63 atoms of Mn<sup>2+</sup> per formula. He has concluded that naturally occurring members of the cumming-tonite-grunerite series vary from 35–100 mole % of Fe<sub>7</sub>Si<sub>8</sub>O<sub>22</sub>(OH)<sub>2</sub> and 0–34 mole% of Mn<sub>7</sub>Si<sub>8</sub>O<sub>22</sub>(OH)<sub>2</sub> components. The composition of manganooan cummingtonite from Sakarsanahalli exceeds this limit for manganese. Al<sub>2</sub>O<sub>3</sub> and CaO contents in this amphibole are also more than the normal range encountered in cummingtonites. However, the Z-position is nearly filled by Si. The CaO content of 2.02 wt% (0.32 atoms per formula) indicates that Ca is more soluble in manganooan than Mn-poor cummingtonite.

The reason for such a higher concentration of manganese in this amphibole is the Mn-rich environment under which it is formed. This is indicated by the manganese content in the associated carbonates (Table II). They are, in general, (Ca, Mn, Mg) CO<sub>3</sub> with MnO content varying from 12 to 25 wt%, corresponding to manganooan calcite to kutnohorite compositions. This is further shown by the manganese content in the coexisting tremolite with 0.81 and 1.16 atoms per formula. In tremolites, Mn<sup>2+</sup> substitutes for Fe<sup>2+</sup> in the Y-positions since the bigger calcium ions occupy the M<sub>4</sub> sites.

## POWDER DIFFRACTION PATTERN

Powder diffraction pattern for the manganooan cummingtonite is obtained with a Philips camera of 114.6 mm diameter, using Cr K <sub>$\alpha$</sub>  radiation (V-filtered) from a Rich-Seifert X-ray unit. In order to obtain the weak reflections, the film is exposed for 48 hours. Measurements are made to an accuracy of 0.05 mm and the film shrinkage is found to be lower than this. The 0 12 0 reflection is used to get the *b* value while 310, 061 and 20 $\bar{2}$  reflections are used to extract *a* sin  $\beta$ , *c* sin  $\beta$ , and  $\beta$  values. This results in the following cell dimensions: *a* = 9.598 Å, *b* = 18.072 Å, *c* = 5.266 Å,  $\beta$  = 103° 44'. The cell volume is 887.2 Å<sup>3</sup> and a calculated density of 3.34 g/cc. The measured density is 3.26 g/cc. Using the cell parameters, the observed reflections in the powder diffraction pattern are indexed (Table III). The agreement between calculated and the observed *d*-spacing indicates the accuracy of the cell parameters.  $\beta$ -value is intermediate between cumming-

TABLE III

X-ray powder diffraction data of manganooan cummingtonite

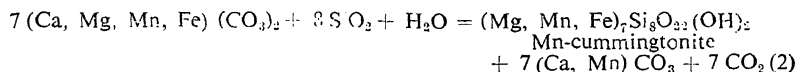
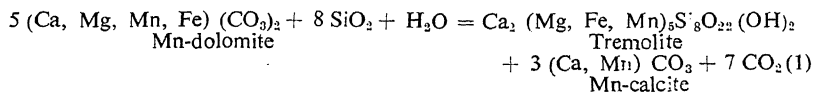
$a = 9.598 \text{ \AA}$ ,  $b = 18.072 \text{ \AA}$ ,  $c = 5.266 \text{ \AA}$  ( $\pm 0.005 \text{ \AA}$ ),  
 $\beta = 103^\circ 44'$ ,  $V = 887.2 \text{ \AA}^3$ ,  $Z=2$ , sp.-group,  $C_{2/m}$

| $d(\text{\AA})$ | $hkl$ | Intensity | $d(\text{\AA})$ | $hkl$  | Intensity |
|-----------------|-------|-----------|-----------------|--------|-----------|
| 9.02            | 020   | 50        | 1.855           | 510    | 10        |
| 8.28            | 110   | 75        | 1.782           | 530    | 3         |
| 4.512           | 040   | 3         | 1.698           | 372    | 5         |
| 4.140           | 220   | 10        | 1.684           | 551    | 3         |
| 3.384           | 131   | 5         | 1.640           | 461    | 50        |
| 3.241           | 240   | 50        | 1.617           | 1 11 0 | 30        |
| 3.059           | 310   | 100       | 1.554           | 600    | 15        |
| 2.932           | 221   | 5         | 1.505           | 0 12 0 | 20        |
| 2.761           | 330   | 10        | 1.495           | 602    | 8         |
| 2.712           | 151   | 25        | 1.452           | 3 11 0 | 5         |
| 2.595           | 061   | 10        | 1.431           | 4 10 1 | 3         |
| 2.508           | 202   | 8         | 1.409           | 661    | 90        |
| 2.352           | 350   | 5         | 1.357           | 512    | 5         |
| 2.293           | 171   | 15        | 1.343           | 1 11 2 | 8         |
| 2.265           | 421   | 10        | 1.338           | 662    | 3         |
| 2.191           | 242   | 3         | 1.298           | 0 12 2 | 5         |
| 2.160           | 261   | 15        | 1.290           | 0 14 0 | 10        |
| 2.047           | 202   | 5         | 1.281           | 751    | 5         |
| 2.008           | 351   | 5         | 1.207           | 602    | 3         |
| 1.959           | 281   | 8         | 1.185           | 5 11 2 | 10        |

tonite ( $102^\circ$ ) and tremolite ( $105^\circ$ ). The smaller  $b$ - and  $c$ -values of the manganooan cummingtonite are nearer to that of tremolite. The  $a$ -axis, however, remains unchanged. The linear plots of  $a \sin \beta$  and of  $b$ -values against composition in the Fe-Mg series will not hold good for the Mg-Mn-Fe ternary cummingtonites. The intensity ratios of the observed reflections also are at variance from those of Mn-free cummingtonite.

#### DISCUSSION

In the silicate-carbonate horizons, tremolite and cummingtonite are formed by the reaction of manganooan dolomite with silica impurities during metamorphism as per the reactions:



The Ca/Mg+Mn+Fe ratio in the dolomite is more than that of tremolite and that of cummingtonite. Therefore manganooan calcite becomes a product. The coexisting carbonates have all the characteristics of high temperature variety and are crystalline. Cummingtonite and tremolite thus formed will incorporate considerable amounts of

manganese, the extent of which is limited by the crystal chemistry of the silicates.

In the amphibole structure, bigger Ca-ions occupy the  $M_4$  sites with an eight-coordination while the smaller cations show preference to  $M_1$ ,  $M_2$  and  $M_3$  sites which have distorted octahedral coordination. This results in the tremolite and hornblende structures<sup>8</sup>. On the other hand, in cummingtonite, all the four sites,  $M_1$  to  $M_4$  have distorted octahedral coordination. The dual coordination of  $M_4$  sites in clinoamphiboles is possible due to the noncoplanar configuration of the oxygens forming the bases in the silicate double chains. The  $(\text{Si}_4\text{O}_{11})$  chains can be bent about the (001) axis, modifying the nature of their stacking. Accordingly, the  $\beta$  adopts extreme values. The  $M_4$  sites can thus acquire any coordination between six and eight. In the Mg-Fe-Mn cummingtonite, containing more than two  $\text{Mn}^{2+}$  per formula, can have manganese filling in the  $M_1$  or  $M_3$  sites, over and above the  $M_4$  occupancy. In such cases,  $\text{Mn}^{2+}$  in the  $M_4$  site gains eight-coordination, with a higher ionic radius ( $1.01 \text{ \AA}$  for the high-spin state)<sup>9</sup>, the silicate chains suitably modify and the structure approaches that of tremolite. This accounts for the closer similarity in physical properties of manganooan cummingtonite and tremolite and so also the cell parameters. Besides, Ca has higher solubility in Mn-cummingtonite than in Mn-free variety which supports the above conclusion. However, due to crystal field effects, the solid solubility between Mn-cummingtonite and tremolite can be expected to be limited.

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## DIMETHYL SULPHOXIDE COMPLEXES OF RARE-EARTH BROMIDES

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## ABSTRACT

Dimethyl sulphoxide (DMSO) complexes of six rare-earth bromides of the composition  $M(\text{DMSO})_8\text{Br}_2$ , where  $M = \text{La, Pr, Nd, Sm, Ho and Y}$  have been prepared and characterized. Infrared studies of the complexes indicate that dimethyl sulphoxide coordinates to metal through oxygen. Conductivity data show a coordination number of ten for all rare-earths. The relative effect of halide ions on the coordination number of the rare-earth ions is discussed.

## INTRODUCTION

**D**IMETHYL sulphoxide complexes of rare-earth chlorides<sup>1</sup>, nitrates<sup>2</sup> and perchlorates<sup>3</sup> have been reported from this laboratory. In all these cases a coordination number of eight for the lighter lanthanides and seven for the heavier lanthanides and yttrium has been suggested from the conductance data. But from the crystal and molecular structure studies of dimethyl sulphoxide complexes of lanthanum nitrate<sup>4</sup> and ytterbium nitrate<sup>5</sup>, it has been shown that the coordination numbers for lanthanum and ytterbium should be ten and nine respectively. Recently rare-earth iodide complexes of dimethyl sulphoxide have been prepared and characterized in this laboratory<sup>6</sup>. A coordination number of nine for all the rare-earths has been suggested in these complexes from the conductivity data. So far very little work has been carried out on the effect of the bromide ion on the coordination number of the rare-earths. In the present paper, for the first time, the isolation and characterization of rare-earth bromide complexes with dimethyl sulphoxide are described.

## EXPERIMENTAL

**Materials.**—The hydrated rare-earth bromides were prepared from pure (99.9%) rare-earth oxides and hydrobromic acid.

Water white DMSO (Crown Zellerbach Co., U.S.A.) was used for the preparation of the complexes.

The commercial dimethylformamide (DMF) was shaken with KOH pellets for two hours and distilled under reduced pressure. The middle fraction distilling at 70° C at 10 mm pressure was collected and its specific conductance was  $9.1 \times 10^{-7} \text{ ohm}^{-1} \text{ cm}^{-1}$  at 30° C.

Acetonitrile was purified by the standard method<sup>7</sup>. Its specific conductance was  $2.63 \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^{-1}$ .

**Preparation of the Complexes.**—One gm. of the hydrated bromide was dissolved in 2 ml of DMSO and the excess of the ligand was removed under reduced pressure. The solid crystalline product

was washed with dry benzene to free it from DMSO and dried.

**Analyses.**—The complexes were analysed for their metal, bromide and DMSO parts. The metal content was estimated by EDTA titrations using xylenol orange as the indicator<sup>8</sup>. The bromide was estimated by Volhard's method and the DMSO by oxidation with excess of permanganate<sup>9</sup>. Since bromide is also oxidised by permanganate, it was removed as silver bromide by adding silver nitrate.

**Infrared Spectra.**—The infrared spectra of the complexes were taken in both nujol mulls and KBr pellets employing a Carl Zeiss UR-10 infrared spectrophotometer. The mull and KBr pellet spectra of the complexes were almost identical. The important absorption bands in the region 3600–600  $\text{cm}^{-1}$  and their assignments, similar to those given for other lanthanide-DMSO complexes, are presented in Table II.

**Conductivity Measurements.**—The molar conductances of the complexes in acetonitrile, dimethylformamide and water were determined with a Siemens conductivity bridge using an immersion cell (Cell constant 0.665). The concentration of the solutions was ca. 0.001 M.

**Molecular Weight Measurements.**—The molecular weights for some representative complexes in water were determined by the freezing point depression method. However, molecular weight measurements could not be carried out in organic solvents because of their poor solubility in suitable solvents. Molecular weight and conductance data are presented in Table III.

## RESULTS AND DISCUSSION

Analytical results (Table I) show that the complexes have the formula  $M(\text{DMSO})_8\text{Br}_2$  where  $M = \text{rare-earth metal}$ . Unlike the corresponding perchlorate complexes, the metal : ligand ratio is eight for all the lanthanides and yttrium. However, the composition is similar to that of the iodide complexes.

The complexes are very hygroscopic, insoluble in non-polar solvents and acetone, and soluble in

TABLE I  
Analytical data for the complexes

| Compound                               | Metal (%) |       | Bromide (%) |       | DMSO (%) |       |
|--|-----------|-------|-------------|-------|----------|-------|
|  | calc.     | found | calc.       | found | calc.    | found |
| La (DMSO) <sub>8</sub> Br <sub>3</sub> | 13.87     | 13.74 | 23.93       | 23.89 | 62.23    | 62.05 |
| Pr (DMSO) <sub>8</sub> Br <sub>3</sub> | 14.03     | 14.04 | 23.84       | 23.58 | 62.10    | 62.50 |
| Nd (DMSO) <sub>8</sub> Br <sub>3</sub> | 14.32     | 14.42 | 23.82       | 23.43 | 61.93    | 61.65 |
| Sm (DMSO) <sub>8</sub> Br <sub>3</sub> | 14.84     | 14.77 | 23.67       | 23.39 | 61.58    | 61.16 |
| Ho (DMSO) <sub>8</sub> Br <sub>3</sub> | 16.03     | 16.05 | 23.31       | 23.20 | 60.66    | 60.53 |
| Y (DMSO) <sub>8</sub> Br <sub>3</sub>  | 9.43      | 9.42  | 24.33       | 24.22 | 66.24    | 65.95 |

TABLE II  
The principal absorption bands in the I.R. spectra of DMSO and its rare-earth bromide complexes (in cm<sup>-1</sup>)

|  | S = O stretch | CH <sub>3</sub> -rocking | Asym C—S stretch |
|--|---------------|--------------------------|------------------|
| DMSO*                                  | 1042 vs       | 962 s<br>905 w           | 695 m            |
| La (DMSO) <sub>8</sub> Br <sub>3</sub> | 1016 vs       | 966 s<br>914 w           | 720 m            |
| Pr (DMSO) <sub>8</sub> Br <sub>3</sub> | 1016 vs       | 964 s<br>909 w           | 718 m            |
| Nd (DMSO) <sub>8</sub> Br <sub>3</sub> | 1016 vs       | 966 s<br>910 w           | 718 m            |
| Sm (DMSO) <sub>8</sub> Br <sub>3</sub> | 1018 vs       | 964 s<br>912 w           | 720 m            |
| Ho (DMSO) <sub>8</sub> Br <sub>3</sub> | 1015 vs       | 965 s<br>916 w           | 720 m            |
| Y (DMSO) <sub>8</sub> Br <sub>3</sub>  | 1012 vs       | 964 s<br>914 w           | 718 m            |

\* Spectrum of DMSO was taken in the liquid phase. Spectra of the complexes taken in nujol mulls are presented.

Abbreviations: vs = very strong; s = strong; m = medium; w = weak.

polar solvents. The colours of the complexes are similar to those of the corresponding salts.

The molar conductances of the complexes in water and DMF are in good agreement with those reported for 1:3 and 1:2 electrolytes respectively<sup>10,11</sup>. The molar conductances in acetonitrile, however, show that the complexes behave as 1:1 electrolytes<sup>11</sup> which suggest the possibility of two of the bromide ions being coordinated to the rare-earth ions. Thus a coordination number of ten can be postulated for all the rare-earth ions. This is the only example where a coordination number of

ten is suggested for a heavier lanthanide and yttrium in DMSO complexes, whereas for lighter lanthanides and coordination number of 10 has been postulated in rare-earth nitrate-DMSO complexes from crystal structure studies<sup>4</sup>.

The molecular weight data for the complexes in water show the presence of twelve species thereby suggesting the complete dissociation of the complexes in water.

The infrared spectra of all the complexes are similar showing no significant dependence on the central metal ion. The intense band occurring at



TABLE III  
Conductance and molecular weight data

| Compound                               | Molar conductance<br>(ohm <sup>-1</sup> cm <sup>2</sup> mole <sup>-1</sup> ) in |       |                    | Molecular weight in water |       |                |
|--|---|-------|--------------------|---------------------------|-------|----------------|
|  | Water   | DMF   | CH <sub>3</sub> CN | obtd.                     | calc. | No. of species |
| La (DMSO) <sub>8</sub> Br <sub>3</sub> | ..  | 159.7 | 91.88              | ..                        | ..    | ..             |
| Pr (DMSO) <sub>8</sub> Br <sub>3</sub> | 400.2   | 160.6 | 95.00              | 80.83                     | 1005  | 12             |
| Nd (DMSO) <sub>8</sub> Br <sub>3</sub> | 410.0   | 167.5 | 99.33              | ..                        | ..    | ..             |
| Sm (DMSO) <sub>8</sub> Br <sub>3</sub> | 403.4   | 163.6 | 94.71              | 84.14                     | 1014  | 12             |
| Ho (DMSO) <sub>8</sub> Br <sub>3</sub> | 411.4   | 164.1 | 96.00              | 84.76                     | 1029  | 12             |
| Y (DMSO) <sub>8</sub> Br <sub>3</sub>  | ..  | 164.7 | 92.94              | ..                        | ..    | ..             |

1045 cm<sup>-1</sup> attributable to the S=O stretch<sup>12</sup> in free DMSO shifts to about 1016 cm<sup>-1</sup> in the complexes, demonstrating the involvement of oxygen in the coordination. An enhancement in the C-S stretching fundamental (from 695 cm<sup>-1</sup> to about 718 cm<sup>-1</sup>) further confirms the oxygen coordination<sup>1</sup>. Absence of a band at 1045 cm<sup>-1</sup> indicates that no occluded or lattice held ligand is present in the complexes.

The extent of S=O shift, which is a measure of the metal-oxygen bond strength in the complexes, is less in bromide complexes (ca. 30 cm<sup>-1</sup>) than in the corresponding rare-earth perchlorate complexes of DMSO (ca. 50 cm<sup>-1</sup>)<sup>3</sup>. These shifts in the rare-earth chloride, bromide and iodide complexes (ca. 35, 30 and 25 cm<sup>-1</sup>) indicate that the metal-oxygen bond strength decreases as we go from chloride to iodide complexes. From the data obtained, the rare-earth bromide-DMSO complexes may be represented as [M(DMSO)<sub>8</sub>Br<sub>2</sub>]<sup>+</sup>Br<sup>-</sup>.

As pointed out in literature<sup>13</sup>, the number of ligands coordinated to the metal ion is dependent upon the anion in the complex. In the case of rare-earth chloride-DMSO complexes the maximum number of ligands coordinated to the metal ion is four, while in the case of the bromide complexes it is eight. In the rare-earth iodide complexes, however, the number of DMSO molecules coordinated to the metal ion remains eight as in the case of bromide complexes.

For the lanthanide ions, the coordination affinities of halide ions lie in the sequence F<sup>-</sup> > Cl<sup>-</sup> > Br<sup>-</sup> > I<sup>-</sup><sup>14</sup>. Hence it is to be expected that rare-earths will add on more number of ligands in bromide complexes than in chloride ones. However, in the case of iodide complexes the number of ligands coordinated

to the metal ion is not further enhanced, probably due to the large ionic size of iodide ion. The interesting feature of the bromide complexes is that the lanthanides have a higher coordination number, as the number of anions coordinated to the metal ion is two (compared to one in the case of the iodide complexes) and the number of ligands coordinated is also the maximum.

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## A CONTRIBUTION TO THE EMBRYOLOGY OF *UTRICULARIA SCANDENS* OLIVER

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CONSIDERABLE amount of work has been done on the embryology of the genus *Utricularia*<sup>1-7</sup>. The genus *Utricularia* exhibits some interesting features embryologically. The notable among them are the endosperm haustoria and the undifferentiated embryo.

The present account deals with the embryology of *Utricularia scandens* Oliver. The plant is a minute twiner. It is often found growing in association with *Utricularia wallichiana*. The material for present investigation was collected near Hassan (Karnataka State). Flowers and fruits were fixed in F.A.A. After dehydration and paraffin embedding sections were cut at 10–12  $\mu$  and stained with Heidenhein's haematoxylin and eosin.

The flowers are very small, blue in colour, borne on slender scapes. Seeds are minute, reticulate and scrobiculate.

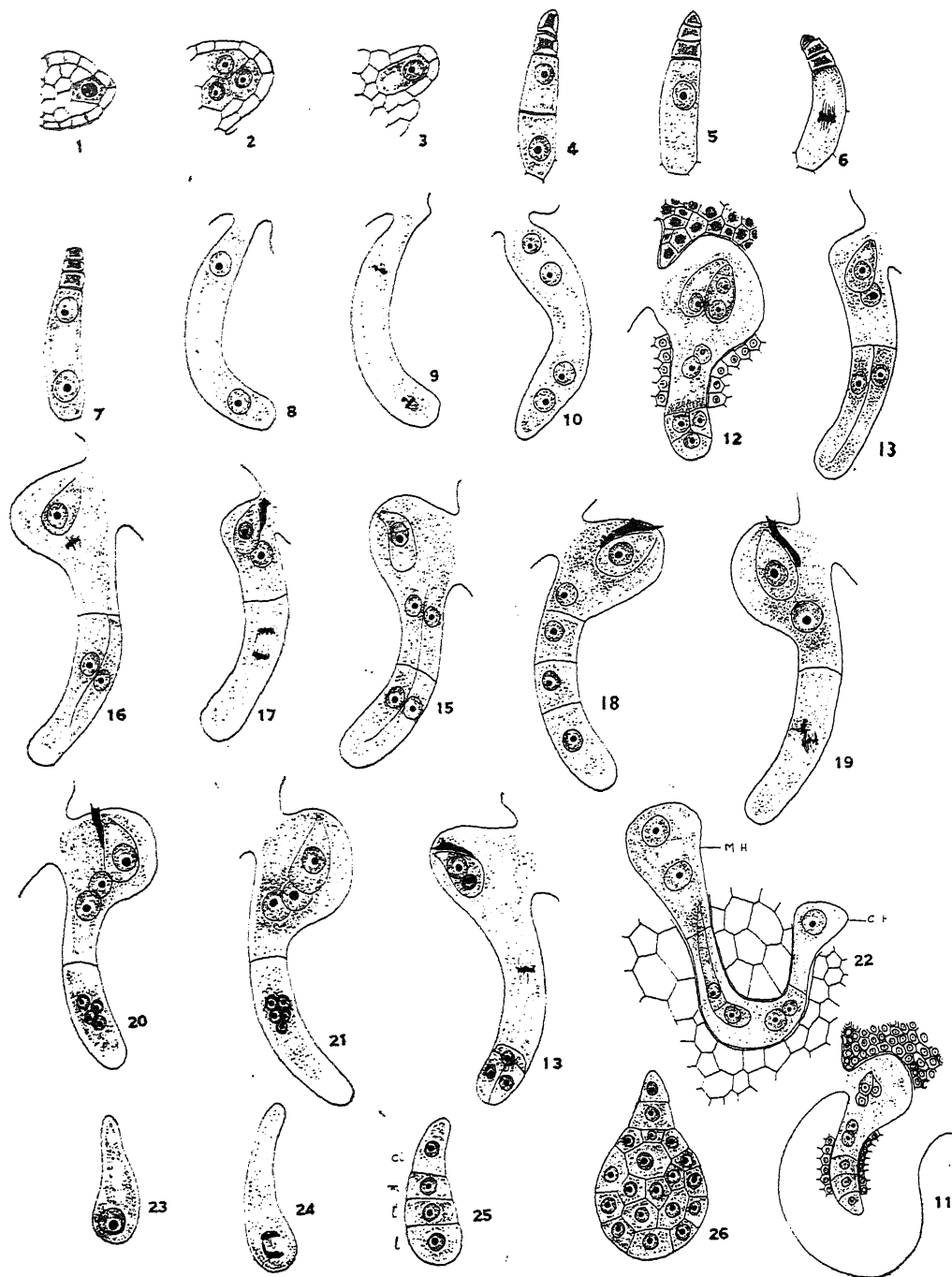
The ovules are anatropous, unitegmic and tenuinucellate. Occasional hemianatropous ovules have been noticed. Placental nutritive tissue is seen at the micropylar part of the ovule (Fig. 11 at corner). A hypodermal archesporial cell arises when the ovule is still erect (Fig. 1). In about 3% of the ovules three archesporial cells have been noticed (Fig. 2). Multiple archesporium is reported earlier in *Utricularia flexuosa* and *Utricularia reticulata*<sup>4-5</sup>. The archesporial cell directly develops into the megaspore mother cell (Fig. 3). In no case more than one megaspore mother cell has been observed. The megaspore mother cell undergoes reduction division and forms a linear tetrad of megaspores (Figs. 5–7). Usually the chalazal megaspore alone is functional and the rest degenerate. But in some ovules two megaspores, i.e., the chalazal two show signs of development (Fig. 4). The functional megaspore divides thrice and develops into an eight-nucleate embryo sac of the Polygonum type (Figs. 8–12). The organised embryo sac is seven-celled (Fig. 12). The embryo sac is extra-ovular. The antipodals are three in number and are organised as cells. The endothelium does not cover the embryo sac completely. It is restricted only to the central region of the embryo sac. Fertilization is porogamous. The first division of the primary endosperm nucleus is transverse to the

long axis of the embryo sac (Fig. 13) and it is followed by the formation of a wall (Fig. 14). The endosperm is *ab initio* cellular. The chalazal cell divides first. The plane of division may be transverse (Fig. 17) or longitudinal (Fig. 16). While this is the usual pattern of division during early endosperm development, interesting variations are observed in some ovules. In some cases, the primary endosperm nucleus divides twice to form four superposed endosperm cells (Fig. 18). In such ovules the middle two cells develop into endosperm proper while the micropylar and chalazal cells develop into haustoria. In very few instances the chalazal endosperm cell becomes coenocytic with a varying number of nuclei (four or five) (Figs. 19–21). The chalazal and micropylar parts of the endosperm develop into haustoria while the middle part develops into endosperm proper. The endosperm haustoria are exactly similar to those of *U. caerulea*<sup>3</sup> (Fig. 22).

The zygote divides only after the initial development of haustoria. The first division of the zygote is transverse (Figs. 23 and 24) resulting in the formation of two superposed cells *ca* and *cb*. Both the cells divide transversely and form a linear pro-embryo of four cells—*l*, *l'* and *ci* and *m* (Fig. 25). *ci* divides once and forms a suspensor of two cells (Fig. 26). The other three cells undergo further divisions and contribute to the embryo proper. The sequence of divisions in the embryogeny follows the Solanad type. The mature embryo is undifferentiated and similar to that seen in *U. caerulea*<sup>3</sup>.

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FIGS. 1-26. Fig. 1. Ovule showing a single hypodermal archesporium. Fig. 2. Ovule showing multiple archesporium. Fig. 3. Megaspore mother cell. Figs. 4-6. Tetrads of megaspores. Figs. 7-12. Stages in the development of embryo sac. Figs. 13-22. Stages in the development of endosperm. Figs. 23-26. Stages in the development of embryo. All figures,  $\times 450$  (except Figs. 11 to 22,  $\times 270$ ).

## LETTERS TO THE EDITOR

### A NOTE ON SUDDEN FIELD ANOMALY PATTERNS (SFA)

ONE of the sudden ionospheric disturbances (SID's) in the sunlit hemisphere, due to the interaction of solar flare produced radiation with the earth's upper atmosphere, is the perturbation in LF field strength (monitored over long paths) designated as Sudden Field Anomaly (SFA). The potentiality of recording the field strength of LF transmissions propagated over long paths as a simple method for solar flare petrol was suggested by Lauter and Sprenger (1958). Experimental investigations of Nestorov (1962), Shirke and Alurkar (1963) and Mitra (1959, 1964) using 164 KHz transmissions from Allouis and Tashkent stations respectively demonstrated this possibility. It is now considered that the LF field strength observation (SFA) is the most efficient method for monitoring and interpreting solar flare effects in the lower ionosphere (Ohle *et al.*, 1974).

Subrahmanyam, Sastri and Deshpande (1974) recently reported the varied nature of the solar flare effect on LF field strength over the Tashkent-Delhi path (path length is about 1600 km) and observed six distinct types of SFA patterns, a typical example of which, with their average size and time structure is shown in Fig. 1. It can be seen that the six SFA patterns can be classified into two categories: Simple and Complex. The

simple SFA patterns are Type II and IV wherein the signal strength either increases (Type II) or decreases (Type IV) following the flare and recovers to the normal level. The complex SFA patterns are Type I, Ia, Ib, and III wherein the signal strength undergoes a series of increases and decreases. The SFA patterns are noticed to exhibit seasonal trends in that Type IV and Ia SFA patterns which are essentially attenuation effects of solar flare, on LF field strength occur mostly in winter and autumn and more or less absent in summer while Type II SFA pattern which is an enhancement effect of solar flare on LF field strength occurs mostly in summer and spring months. One of the interesting characteristics of SFA patterns is that the total duration of simple SFA patterns (Type II and IV) is much less than that of complex patterns (Type I, Ia and III): in fact, the ratio of the latter to the former is of the order of two. This feature can clearly be seen from Fig. 1.

This brief communication is devoted to an understanding of the characteristics of SFA patterns mentioned above, *i.e.*, the slow and relatively gradual recovery nature of field strength in complex patterns compared to simple patterns. The interpretation advanced is that the recovery nature is mainly governed by the range of heights over which the excess flare induced ionization is produced due to X-ray flux enhancement in the 1–20 Å band. Following this argument, the Type II and IV SFA patterns may be understood as due to extra ionization at heights below 60 km and above 75 km respectively [as a result of hardening of the X-ray spectrum (1–20 Å) and soft X-ray flux (1–20 Å) enhancement respectively] whereas the complex SFA patterns (Type I, Ia, III) are due to excess ionization in the height range 60–70 km. Then the recovery nature in complex SFA patterns is governed by the negative ion chemistry of the D-region and the slow recovery nature of the field strength could be due to the slow release of electrons from negative ions by photo-detachment. Support for this interpretation comes from the recent work of Thomas *et al.* (1973), who have studied the role of negative ion changes in the D-region during flare conditions. Using a simulation procedure, with the proportional change in ion pair production rate at each height, represented by an analytical expression (representative of flare effect), with a rise time of 5 min and an

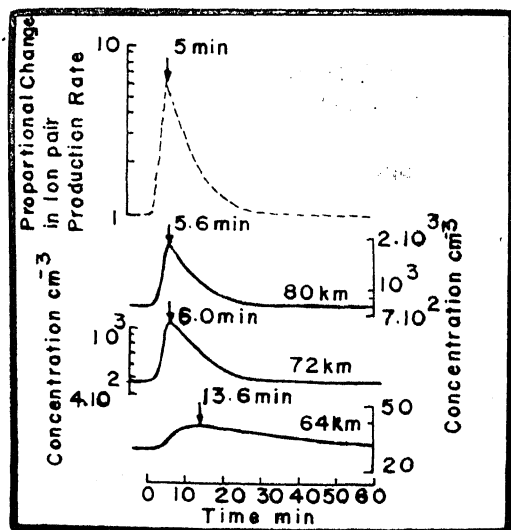


FIG. 1. Typical SFA patterns with their average size and time structure, observed over Tashkent-Delhi path.

exponential decay with a time constant of 5 min they have calculated the variations in concentrations of electrons and negative ions ( $O_2^-$ ,  $NO_3^-$ ,  $CO_3^-$ ) at different heights for two cases: without and with the photo-detachment of electrons from negative ions ( $NO_3^-$ ,  $NO_2^-$ ,  $CO_3^-$ ). They observed that if photo-detachment of electrons from negative ions is taken into consideration, the recovery of electron concentration after its maximum is considerably slowed down and this feature is most striking at a height of 64 km. This can be seen from Fig. 2 reproduced from the paper of Thomas *et al.* (1973).

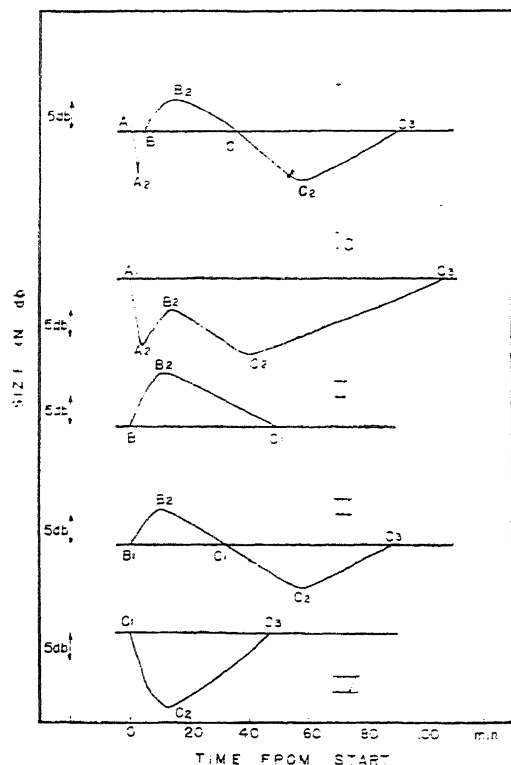


FIG. 2: The proportional change in ion pair production rate at all heights during a flare and the resulting variations of electron concentrations computed for 80, 72, and 64 km in the presence of photodetachment of electrons from ions  $CO_3^-$ ,  $NO_3^-$ ,  $NO_2^-$  according to the rates shown in Table I of Thomas *et al.* (1973) (after Thomas *et al.*, 1973).

It is to be emphasized however that the work of Thomas *et al.* (1973) is based on the assumption that the proportional change in ion pair production rate is the same throughout the D-region, which is an idealised condition. Further work is therefore necessary for a better understanding of the influence of negative ion changes on the recovery

nature of field strength in complex SFA patterns by analysing specific events with the help of X-ray flux data, now available from SOI-RAD satellite.

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Astrophysics,

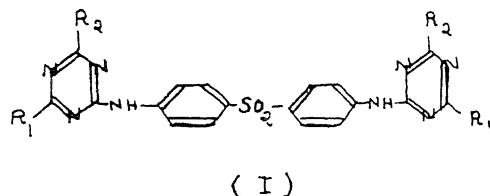
Kodaikanal 624103, December 21, 1974.

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#### ANTIBACTERIAL ACTIVITY OF BIS-*s*-TRIAZINYLAMINOPHENYLSULPHONE

SEVERAL derivatives and analogues of amino sulphones have been shown to have strong tuberculostatic<sup>1</sup>, antileprotic<sup>2</sup> anticonvulsant action and to be of potential use therapeutically. *s*-Triazine nucleus has also been the subject of several investigators in the realm of potential therapeutic agents for diseases like malaria and cancer. Foye and Buckpitt<sup>3</sup> carried out the condensation of cyanuric chloride with diamino thiazolylphenylsulphone in different proportion in order to prepare products of therapeutic use.

We have prepared Bis-*s*-Triazinylaminophenylsulphones of the type (I) with a view to testing them as therapeutic agents.



where  $R_1$  and/or  $R_2$  = chloro, phenylamino, substituted phenylamino.

4-4'-Diaminodiphenylsulphone was condensed with cyanuric chloride at 0° to get *p, p'*-[Bis (2, 4-dichloro-*s*-triazin-6-yl-amino)] diphenyl sulphone which was then condensed with different bases (one mole as well as two moles), *e.g.*, Aniline, *o*-, *m*- and *p*-toluidine at 30–35° and 80–90° respectively using acetone or dioxane as solvent,

### Antibacterial Testing

The following strains were used for testing the antibacterial activity employing agar slants<sup>4</sup>:

A Gram-positive bacterial strains like *Bacillus megaterium*, *Bacillus citreus*, *Bacillus subtilis*, *Staphylococcus aureus*.

B. Gram-negative bacterial strains like *Escherichia coli*, *Aerobacter aerogenes*, *Salmonetta typhi*, *Salmonella paratyphi* A., *Salmonella paratyphi* B., *Shigella dysenteriae sonnei*, *Sh. dy. Shiga*, *Sh. dy. flexneri*, *Sh. dy. schimitazii*, *Pseudomonas aeruginosa*.

Testing was done in nutrient broth. After inoculation with a loopful of culture from the slant, the seeded nutrient broths were incubated at 37° C for 24 hours. The various dilutions of the sulphone derivatives were prepared with a slight modified method as proposed by Dhar *et al.*<sup>5</sup>.

The sulphone derivatives were dissolved in alcohol/acetone to obtain a 10 mg/ml solution. From this solution 0.2 ml was added to 1.8 ml of the above seeded broth which formed the first dilution and contained one mg of sulphone per ml of the seeded broth. One ml of the first dilution was further diluted with one ml of seeded broth to produce the second dilution and so on till five such dilutions were obtained.

From the experiment, it was observed that all the above derivatives are able to inhibit the growth of all Gram-positive bacteria at the 250 µg/ml concentration and all Gram-negative bacteria at the 500 µg/ml concentration.

### Experimental

(a) Preparation of *p-p'* [Bis(2, 4-Dichloro-*s*-triazin-6-yl-amino)]-diphenylsulphone.—4-4'-Diaminodiphenylsulphone (0.01 mole) dissolved in acetone (40 ml) was added to a solution of cyanuric chloride (0.02 mole) in acetone (40 ml) at 0°. Contents were stirred for an hour by simultaneous addition of aqueous sodium hydroxide solution (10 ml, 4%) and diluted with ice water (100 ml). The product was isolated and crystallised. Yield 84.5%, m.p. 360°.

(b) Preparation of *p-p'* [Bis(2-Arylamino-4-chloro-*s*-triazin-6-yl-amino)]-diphenylsulphone.—Bose (0.02 mole) dissolved in dioxane (10 ml) was added to the suspension of *p-p'* [Bis (2, 4-dichloro-*s*-triazin-6-yl-amino)] diphenyl sulphone (0.01 mole) in dioxane (60 ml) at 30–35°. A clear solution was obtained after an hour. Contents were stirred for an hour with the gradual addition of aqueous sodium hydroxide solution (10 ml, 4%) and diluted with ice water (100 ml). The product was isolated and crystallised. The products are as follows. % Yield and m.p. of Anilino : 86.2, 310° (d), *o*-toluidino : 73, 360°, *m*-toluidino : 70, 360° and *p*-toluidino : 72.12, 280°.

(c) Preparation of *p-p'* [Bis (2-4-Diarylamino-*s*-triazin-6-yl-amino)] diphenylsulphone.—Bose (0.02 mole) dissolved in dioxane (10 ml) was added to *p-p'* [Bis (2-Arylamino-4-chloro-*s*-triazin-6-yl-amino)] diphenylsulphone (0.01 mole) dissolved in dioxane (40 ml) and reflux in water bath at 80–85°, for 1½ hour with the simultaneous addition of aqueous sodium solution (10 ml, 4%). Contents were poured into crushed ice (100 g). The product was isolated and crystallised. % Yield and m.p. are as follows : Anilino : 84.00, 290 (d), *o*-toluidino : 69.10, 310°, *m*-toluidino : 73.00, 360°, *p*-toluidino : 77.33, 360°.

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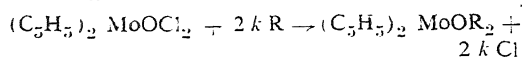
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### PSEUDOHALIDE COMPLEXES OF BISCYCLOPENTADIENYL MOLYBDENUM (VI)

BISCYCLOPENTADIENYL molybdenum (VI) oxydichloride and bisindenyl molybdenum (VI) oxydichloride have already been prepared by treating molybdenum (VI) oxytetrachloride with sodium cyclopentadienide and sodium indenide in tetrahydrofuran<sup>1</sup>. There is no reference in literature regarding the preparation or characterisation of pseudohalide complexes of biscyclopentadienyl molybdenum (VI) and bisindenyl molybdenum (VI) although the preparation and characterisation of metallocene pseudohalide complexes of titanium (IV), titanium (III), zirconium (IV), vanadium (IV) and thallium (III) have been reported<sup>2-8</sup>. Turco and Pecile<sup>10</sup>, Thayer and West<sup>11</sup> and Burmeister<sup>12</sup> have also studied the mode of bonding in certain metal-pseudohalide complexes.

The present communication deals with the preparation and characterisation of pseudohalide complexes of biscyclopentadienyl molybdenum (VI) by the interaction of biscyclopentadienyl molybdenum (VI) oxydichloride with potassium or sodium salts

of pseudohalides in tetrahydrofuran. The products were isolated by removing the solvent under reduced pressure and subsequent crystallisation from petroleum ether.



where R may be CN, NCO, NCS and N<sub>3</sub>  
and k is either potassium or sodium.

The compounds prepared vary from red brown to dark brown in colour and are stable in dry atmosphere. These compounds are non-volatile, soluble in common organic solvents and are hydrolysed by water, dilute acids and alkalis.

**Experimental.**—All the reactions were carried out in dry inert atmosphere. The solvents were dried and purified before use by conventional methods. Tetrahydrofuran was refluxed over potassium hydroxide followed by distillation in presence of lithium aluminium hydride.

To biscyclopentadienyl molybdenum (VI) oxydichloride 1.1 g (0.0035 mole) in tetrahydrofuran (100 ml) was added anhydrous potassium thiocyanate 0.058 g (0.0052 mole). The mixture was refluxed at 70–80° C for 2 hours, cooled and filtered. The filtrate on evaporation under reduced pressure gave red brown residue which on repeated crystallisation from petroleum ether (60–80° C) gave red brown crystals of  $(C_5H_5)_2 MoO(NCS)_2$  yield—80%. Anal. calcd. for  $(C_5H_5)_2 MoO(NCS)_2$ : C, 40.22; H, 2.79; Mo, 26.81%. Found: C, 40.12; H, 2.61; Mo, 26.60%.

Molybdenum was estimated as oxinate and C, H were estimated by microanalytical methods. The i.r. spectra of the compounds recorded on Perkin-Elmer Model-137 Spectrophotometer in KBr medium showed the following absorption peaks:

3010 2050 1710 1540 1470 1160 1050 955 850  
(m) (vs) (m) (w) (m) (w) (m) (m) (s)  
where vs = very strong; s = strong; m = medium;  
w = weak.

The i.r. spectrum of biscyclopentadienyl molybdenum (VI) oxydithiocyanate shows the usual peaks of  $C_5H_5^-$  group, viz., the frequencies at 3010 cm<sup>-1</sup> (C–H stretching), at 1470 cm<sup>-1</sup> (C–C stretching), at 1160, 1050 cm<sup>-1</sup> (C–H in plane bending) and at 850 cm<sup>-1</sup> (C–H out of plane bending). The peak at 960–45 cm<sup>-1</sup> is due to metal-oxygen, i.e., M=O linkage.

Metal thiocyanates (M–SCN) are indicated by C–N stretching vibration appearing at  $\geq 2100$  cm<sup>-1</sup> and C–S stretching vibrations at 690–720 cm<sup>-1</sup>. The metal isothiocyanates (M–NCS) are indicated by C–N stretching at  $\leq 2100$  and C–S stretching at higher frequency 780–860 cm<sup>-1</sup><sup>10</sup>. In cyclopentadienyl compounds, the later band 780–860 cm<sup>-1</sup>

may be masked by the strong absorption around 850 cm<sup>-1</sup> due to C–H out of plane bending vibrations of the ring. However, the absence of any bands in the region 650–750 cm<sup>-1</sup> and the presence of strong bands at 850 cm<sup>-1</sup> and 2050 cm<sup>-1</sup> strongly indicates the presence of M–NCS bonding structure. This is in accord with the observations of Burmeister<sup>12</sup> that N coordination usually results in an increase in C–S and C–N stretching values relative to free ion values (~ 749 cm<sup>-1</sup> and 2060 cm<sup>-1</sup>).

Similar observations were made in i.r. spectra of the metal cyanate showing an intense band at 1320 cm<sup>-1</sup> and this is consistent with the isocyanate structure<sup>11</sup>, i.e., M–NCO. Other pseudohalide complexes were prepared similarly and their analytical and i.r. data was in agreement with the calculated values.

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#### CHEMICAL INVESTIGATION OF THE ESSENTIAL OIL OF PSEUDO- SORGHUM GRASS

THE essential oil (34 g) was separated into acidic and neutral part by treating with an aq. sodium hydroxide solution (5%). Acidic portion was found to contain two components, one giving positive ferric chloride colour test while the other giving effervescence with sodium bicarbonate solution. Therefore, acidic portion was taken in ether and washed with saturated solution of sodium bicarbonate. The sodium bicarbonate insoluble part yielded the solid phenol, i.e., xanthoxylene<sup>2-5</sup> (1.6 g) which was further confirmed by the preparation of its acetyl derivative and the soluble part yielded an

acid, i.e., 3-hydroxy-*p*-toluic acid<sup>6,7</sup> (0.47 g); this too was further confirmed by the preparation of its acetyl derivative.

The neutral part (32 g) was chromatographed over neutral alumina grade II and three fractions were collected by eluting the column with petroleum ether, benzene and ether.

**Petroleum ether fraction:** (TLC five spots) of alumina on extensive chromatography over silicagel and silicagel impregnated with silver nitrate (15%) afforded *n*-tridecane<sup>8,9</sup> (3.5 g), decanone-4<sup>9,10</sup> (0.86 g) and octanone-3 (0.53 g)<sup>11</sup>. All the three compounds have been identified on the basis of their physical, chemical and spectral data.

**Benzene fraction** (TLC, three spots): On extensive chromatography over active silicagel afforded two components, an aromatic ether (under investigation) and an ester, *p*-methoxy methyl cinnamate<sup>12,13</sup>, m.p. 87–88°.

**Ether fraction** (TLC, three spots): On extensive chromatography over active silica gel, afforded an open chain alcohol decanol-4. The structure has been confirmed on the basis of its physical, chemical and spectral data.

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## CHEMICAL EXAMINATION OF THE FLOWERS OF *PEDALIMUM MUREX*

*Pedaliium murex* (Pedaliaceae) is a small flowering plant growing abundantly in the coastal regions of South India. Though there are reports<sup>1</sup> about its therapeutic uses, no detailed examination of the plant as a drug has been done so far. The leaves of this plant have been shown to contain flavonoid compounds<sup>2</sup>. We have now examined the polyphenolic components of the flowers of this plant and report the results in this communication.

The fresh flowers, collected around Madurai University area, were repeatedly extracted with 95% ethanol, till the extract was colourless. The combined extract after concentration was fractionated using neutral and basic lead acetate. The lead salt from the neutral lead acetate fraction after decomposition with hydrogen sulphide yielded a crude mixture of flavonoid compounds.

**Aglycones:** The residue from the decomposition of the neutral lead salt fraction was macerated repeatedly with ether to remove free aglycones and the ether extract after evaporation yielded a mixture of aglycones. Paper chromatography of the aglycone mixture using the B.A.W. as the solvent system (4:1:5, v/v) showed the presence of two flavonoids having  $R_f$  values 0.95 and 0.64. These were separated by preparative paper chromatography. The aglycone having the higher  $R_f$  value was found to be dinatin (5,7,4'-trihydroxy-6-methoxyflavone) by a detailed study of its U.V. spectrum and comparison with an authentic sample. The compound with lower  $R_f$  value was also studied in the same way and identified as quercetin (3,5,7,3',4'-pentahydroxyflavone).

**Glycosides:** The glycoside mixture obtained as the ether insoluble fraction was found by paper chromatography (B.A.W., 4:1:5, v/v) to be a mixture of two compounds ( $R_f$  values 0.32 and 0.28). These were separated by preparative paper chromatography using the same solvent system. The glycoside ( $R_f$  value 0.32) on hydrolysis with 7% sulphuric acid yielded glucose and quercetin (identified by paper chromatography). A detailed study of the U.V. spectra of the glycoside and the aglycone with and without the addition of the various shift reagents suggested its identity as quercetin-7-glucoside. Rigorous comparison of the natural material with authentic quercimeritrin confirmed the identity. The slow-moving component of the glycoside mixture ( $R_f$  value 0.28) on hydrolysis with 7% sulphuric acid yielded rhamnose in addition to quercetin and glucose. By a careful study of the U.V. spectra of the glycoside and aglycone with and without the addition of various reagents such as sodium acetate, sodium ethoxide,

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boric acid and aluminium chloride. it was concluded that both the sugar residues are attached to the oxygen atom at the 7-position of the aglycone.

Further study concerning the sequence of attachment of the sugars and also the identification whether it is a rutinoid or a neohesperidoid is in progress.

Our thanks are due to Prof. S. Sankara Subramanian of the Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-6, for the supply of authentic dinatin used for comparison and to the University Grants Commission, New Delhi, and the Madurai University for the award of a Research Scholarship to one of us (S. M. K.).

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### EFFECT OF MINERAL NUTRITION ON YIELD AND OIL QUALITY OF SUNFLOWER

EXPERIMENTAL data on the effects of mineral nutrition on the yield and oil content of sunflower, a recently introduced oil seed crop, are available in literature<sup>2-5</sup> but the role of mineral nutrient uptake on oil quality has not been studied. This aspect was investigated on a sandy loam soil at Agricultural College Farm, Bapatla, with two varieties of sunflower, viz., Sunrise and E.C. 68414.

The layout was a split-plot design, with the varieties being assigned to main plots and three dates of sowing combined with four levels of phosphate (0, 30, 60 and 90 kg  $P_2O_5$ /ha as superphosphate) being assigned to sub-plots: there were three replications. The crop received uniform dose of 60 kg N/ha as ammonium sulphate and 30 kg  $K_2O$ /ha as muriate of potash. In addition to the yield of seed, oil-yield was calculated from the per cent oil/in seeds estimated with Soxhlet's extraction apparatus (A.O.A.C., 1970). Attributes of oil quality were determined by standard procedures.

Data on the (maximum) uptake of major nutrients N, P and K at complete flower-opening as well as yield and oil quality attributes are given in Table I.

The variety Sunrise gave significantly higher yields of seed and oil as compared to E.C. 68414. For both the varieties the uptake of K was highest followed by N and P, though there were no differences in the uptake of each nutrient between the two varieties. Similarly, there were no marked variations in the acid value, saponification value and iodine value of oil for the two varieties.

The relation between the uptake of N, P and K at complete flower-opening and the yield as well as oil quality attributes are given in Table II.

Uptake of N, P and K at complete flower-opening were significantly correlated with both seed and oil yields, those with oil-yield being still higher. The acid value and iodine value bore significant correlation with uptake of N and K only but not with that of P. Nutrient uptake did not seem to influence saponification value of oil. Saponification value which is indicative of the presence of long chain fatty acid components seems to be a characteristic

TABLE I

*Uptake of major nutrients at complete flower opening and yield and oil quality of sunflower*

| Variety       | Uptake of nutrients<br>in mg per plant |      |       | Yield in<br>kg/ha |       | Quality characteristics<br>of oil |                           |                 |
|---------------|--|------|-------|-------------------|-------|-----------------------------------|---------------------------|-----------------|
|               | N                                      | P    | K     | Seeds             | Oil   | Acid<br>value                     | Saponifica-<br>tion value | Iodine<br>value |
| 1. Sunrise    | 320.2                                  | 69.8 | 793.4 | 1334.2            | 504.2 | 6.04                              | 184.5                     | 122.0           |
| 2. E.C. 68414 | 323.0                                  | 68.7 | 795.3 | 1267.6            | 483.8 | 6.02                              | 183.8                     | 121.7           |
| Significance  | N.S.                                   | N.S. | N.S.  | Yes               | Yes   | ..                                | ..                        | ..              |
| CD at 5%      | ..                                     | ..   | ..    | 6.32              | 7.45  | ..                                | ..                        | ..              |

N.S. = Not significant.

TABLE II

Correlation of nutrient uptake at complete flower-opening with yield and quality of sunflower oil

| Correlation between | Yield of seeds | Yield of oil | Acid value | Saponification value | Iodine value |
|---------------------|----------------|--------------|------------|----------------------|--------------|
| Uptake of N         | 0.82**         | 0.89**       | 0.43*      | 0.19                 | 0.52**       |
| Uptake of P         | 0.62**         | 0.91**       | 0.32       | 0.35                 | 0.23         |
| Uptake of K         | 0.65**         | 0.85**       | 0.62**     | 0.15                 | 0.57**       |

\* Significant at 5%.

\*\* Significant at 1%.

of plant species, and is not so much influenced by nutrient uptake.

The present investigation revealed that sunflower variety Sunrise was superior to E.C. 68414 as regards the yield of seed and oil, though the uptake of N, P and K by the varieties did not differ much. The uptake of N, P and K at complete flower-opening was found to bear significant positive correlation with both seed and oil yield. The acid value and iodine value appeared to be affected by the uptake of N and K but not of P. Saponification value was not influenced by nutrient (N, P or K) uptake at complete flower-opening.

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### ISOCITRATE DEHYDROGENASE IN RHIZOBIUM SPECIES

NICOTINAMIDE adenine dinucleotide phosphate may be a key electron carrier from which electrons are channelled to nitrogenase in *Azotobacter*<sup>1</sup>, nodule bacteroids<sup>2</sup> and blue green algae<sup>3</sup> from the general metabolic pool. A system generating NADPH could be coupled to bacteroid nitrogenase with bacteroid iron-sulphur proteins azotoflavin and ferredoxin NADP reductase<sup>4</sup>. The role of poly-beta-hydroxybutyrate dehydrogenase in rhizobia, which is linked to pyridine nucleotides, has been well studied<sup>5-6</sup>. The general dehydrogenase level

in various effective and ineffective strains has been reported<sup>7</sup>.

Isocitrate dehydrogenase (ICD) constitutes 1% of the total protein in *Azotobacter vinelandii* and its role in supplying NADPH (electrons) to nitrogenase has been suggested<sup>8</sup>. The enzyme has been reported in *Rhizobium* bacteroids<sup>2</sup>. However, cultured rhizobia produce glutamate dehydrogenase and aspartate and alanine aminotransferases but not isocitrate dehydrogenase, when given ammonia or amino acids<sup>9</sup>. Since very low ICD activity was detected in bacteroids, the supply of  $\alpha$ -ketoglutarate by the host plant during symbiosis has been suggested<sup>10</sup>. We report here the presence of ICD in all of the effective and ineffective species of rhizobia cultured *in vitro*.

*Rhizobium* strains were grown in a medium containing  $K_2HPO_4$  1.6 g,  $KH_2PO_4$  0.4 g, Yeast Extract 2 g,  $MgSO_4 \cdot 7H_2O$  0.2 g, NaCl 0.1 g,  $CaCl_2 \cdot 2H_2O$  0.09 g,  $(NH_4)_2SO_4$  0.5 g and Sucrose 20 g per litre in 500 ml Erlenmeyer flasks with 200 ml medium. A 10% inoculum was added and the flasks were incubated on a rotary shaker at 30°C. Cells were harvested during the late logarithmic phase and washed twice with 0.05 M phosphate buffer. Crude extracts were prepared by grinding the frozen cell paste with two parts of glass powder followed by extraction with 4 volumes of 0.005 M Tris-HCl buffer of pH 7.5. Unbroken cells and debris were removed by centrifugation at 4°C at 20,000  $\times$  g and the supernatant used for enzyme assays.

The enzyme was assayed spectrophotometrically by measuring the rate of reduction of NADP at 340 nm in presence of the substrate. The assay mixture contained DL-isocitrate (trisodium salt) 20  $\mu$ moles, NADP 0.5  $\mu$ moles,  $MnCl_2$  10  $\mu$ moles, Tris-HCl buffer pH 7.5, 40  $\mu$ moles. The reaction was started by addition of substrate<sup>11</sup>. Extinction was measured in a Beckman DU spectrophotometer.

Protein was estimated by the modified folin's method<sup>12</sup>. One unit is that amount of enzyme which catalyses the formation of 1  $\mu$ mole of reduced NADP per minute. Specific activity is expressed as units per mg protein.

Contrary to earlier reports, all strains of *Rhizobium* tested showed ICD activity (Table I).

TABLE I

| Strain                              | Description   | Specific activity of ICD (units/mg) |
|-------------------------------------|---|-------------------------------------|
| Obtained from C.S.I.R.O., Australia |   |                                     |
| <i>Rhizobium japonicum</i> CB 1809  | Effective for all varieties of <i>Glycine max</i> except Hardee and related lines | 0.18                                |
| <i>R. japonicum</i> CC 709          | Effective for <i>Glycine max</i>  | 0.07                                |
| <i>R. japonicum</i> CC 707          | Ineffective for <i>Glycine max</i>  | 0.061                               |
| <i>R. phaseoli</i> CC 502           | Effective for <i>Phaseolus vulgaris</i>   | 0.05                                |
| <i>R. phaseoli</i> CC 596           | Ineffective for <i>Phaseolus vulgaris</i>   | 0.051                               |
| Isolated in our laboratory          |   |                                     |
| <i>R. phaseoli</i>                  | Effective for <i>P. vulgaris</i>  | 0.13                                |

The level of the enzyme differs from species to species and there is no significant difference between the effective and ineffective strains. Differences in the cultural conditions used in the growth of *Rhizobium* could account for the inability of earlier workers to detect ICD.

We are indebted to Professor F. J. Bergerson, C.S.I.R.O., Australia, for the cultures of *Rhizobia*. The senior author thanks the C.S.I.R., New Delhi, for the award of a Junior Research Fellowship.

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#### EPIZOOTIC SEPTICEMIA IN FROGS CAUSED BY *AEROMONAS HYDROPHILA*

AN investigation was carried out to determine the cause of the sudden death in the frog-tank of frogs in large numbers at this college (Fig. 1). The

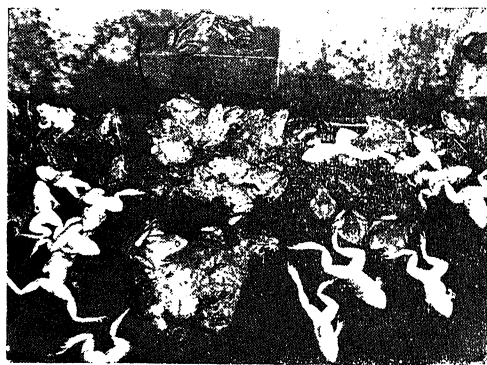


FIG. 1. Photograph showing the dead frogs in the frog tank.

diseased frogs were very lethargic, and "punched" ulcers, over the knees, thighs, back and nostrils were seen. On application of pressure near the lesions, a straw coloured fluid exuded from the ulcers. The webs were bright red in colour. Few diseased frogs, tank-deposit, and the tank water were collected for bacteriological investigation. Scrapings from the ulcers, heartblood, deposit from

tank and water were inoculated in different media. *Aeromonas hydrophila* was isolated from all the above specimens to the exclusion of other pathogenic bacteria and fungi. All the isolates were oxidase positive and produced very clear halos of beta hemolysis on blood agar plates; they gave non-lactose fermenting colonies on MacConkey agar plates. The nature and characteristics were identical to that of *Aeromonas hydrophila* as described in the *Bergey's Manual of Determinative Bacteriology*<sup>3</sup>. Therefore it was identified as *Aeromonas hydrophila*.

*Aeromonas hydrophila* of the family Pseudomonadaceae is a common inhabitant of water as the very name suggests. It can also be isolated from soil, foods and rarely from the human intestinal tract<sup>2</sup>. It is pathogenic for frogs ("Red-leg" disease), salamanders, fish, mice, guinea pigs, rabbits and snakes causing hemorrhagic septicemia<sup>3,5</sup>, abortion in bovidae<sup>6</sup>, and 'black rot' in hen's eggs<sup>3</sup>. In our laboratory a tortoise also died of this infection but this animal was kept in a separate aquarium. There are a few reports of human infection caused by *Aeromonas hydrophila* like septicemia<sup>1</sup>, gastroenteritis<sup>2,4</sup>, cellulitis and cirrhosis<sup>2</sup>. One of the authors (K. N. A.) who handled these frogs with bare hands developed a 'Whitlow' in three to four days time presumably due to the same organisms. The pus smear showed gram negative bacilli.

According to the authors, so far, there is no report from India of the outbreak of "Red-leg" in frogs. This communication records an epidemic, the etiological agent clearly being *Aeromonas hydrophila*.

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## ON AN OCCURRENCE OF HIDDENITE FROM A PEGMATITE NEAR KABBUR, HASSAN DISTRICT, MYSORE STATE

THE paper describes the occurrence of a rare variety of spodumene, from a mica-pegmatite exposed at Kabbur. Rao and Rao<sup>1</sup> and Babu<sup>2</sup> have reported the occurrence of spodumene in India. The pegmatites occurring at Kabbur, are essentially mica bearing, belonging to Archaean age. The pegmatite is found traversing and sometimes associated with granitic gneisses. The pegmatite is generally coarse-grained, consisting of quartz, feldspar and mica. Both types of micas are seen in the pegmatite. Muscovite occurs in books of varying size, while biotite occurs sporadically interspersed in the pegmatite and occurs as streaks and irregular books. Garnet and black tourmaline occasionally are seen in the pegmatite.

The author collected a few crystals from the mines. The pegmatite, containing the spodumene crystals comprises highly crushed quartz and lustrous plates of feldspars. The pegmatite containing hiddenite is even textured and the microcline in the pegmatite is devoid of perthitic habit.

The mineral was crushed to pass through 80 mesh and the crushed material was subjected to heavy liquid separation. The material separated was almost pure but for the inclusions inside the mineral, which could not be separated. X-ray and optical data confirmed the identification of the mineral. The data on chemical analysis and X-ray are presented in Table I.

The crystal collected from the pegmatite dumps, was tabular, measuring 2" in length, with vertical furrows and striae. Very well developed one set of prismatic cleavage, exhibiting pearly lustre on the cleavage surface are seen. The colour ranges from emerald-green to apple green. On some portions of the crystal collected from the crushed pegmatite portion, alteration into a soft, yellowish-green to pale pink material is seen which may be cymatolite(?), as determined by the lithia content (0.20%).

*Chemical Study.*—The material separated in heavy liquid was chemically analysed. The major analysis was carried out after the method of Schapiro and Brannock<sup>3</sup> and the trace elements by the use of Atomic absorption spectrometer (Cu, Mg, Ca, Sr, Rb) and Spectrophotometer (Cr, Ti) and Emission Spectrograph (Be, B). The major and trace elemental analyses are presented along with the analysis reported in literature in Table I.

The chemical analysis compares well with the purplish-grey to greenish spodumene reported in Deer et al.<sup>4</sup> (p. 93). The mineral from Kabbur

TABLE I

## X-ray Data for Hiddenite

| 2 $\theta$ value<br>(In<br>degree) | 'd'<br>value | I I <sub>0</sub> | 2 $\theta$<br>value | 'd'<br>value | I I <sub>0</sub> |
|------------------------------------|--------------|------------------|---------------------|--------------|------------------|
| 20                                 | 4.439        | 50               | 44.62               | 2.031        | 10               |
| 21.17                              | 4.196        | 60               | 47.17               | 1.927        | 20               |
| 25.89                              | 3.446        | 40               | 48.88               | 1.863        | 50               |
| 28.02                              | 3.184        | 40               | 49.38               | 1.845        | 5                |
| 29.31                              | 3.047        | 10               | 52.67               | 1.738        | 10               |
| 30.66                              | 2.916        | 100              | 55.76               | 1.649        | 5                |
| 31.30                              | 2.858        | 10               | 58.92               | 1.567        | 60               |
| 32.06                              | 2.792        | 75               | 60.75               | 1.525        | 30               |
| 33.61                              | 2.666        | 20               | 63.75               | 1.460        | 40               |
| 36.69                              | 2.450        | 60               | 66.94               | 1.398        | 20               |
| 38.28                              | 2.351        | 20               | 70.90               | 1.329        | 30               |
| 40.65                              | 2.219        | 10               | ..                  | ..           | ..               |
| 42.18                              | 2.142        | 8                | ..                  | ..           | ..               |
| 42.95                              | 2.106        | 40               | ..                  | ..           | ..               |
| 44.03                              | 2.057        | 20               | ..                  | ..           | ..               |

## Chemical data of Hiddenite

| Constituents                   | A      | C      | Trace elements | In (ppm) |
|--------------------------------|--------|--------|----------------|----------|
| SiO <sub>2</sub>               | 63.90  | 64.16  | Ct             | 1200     |
| Al <sub>2</sub> O <sub>3</sub> | 26.82  | 27.74  | Rb             | 150      |
| Fe <sub>2</sub> O <sub>3</sub> | 0.41   | 1.03   | Mg             | 200      |
| FeO                            | 0.70   | ..     | Ca             | 50       |
| MnO                            | 0.24   | 0.32   | Sr             | 10       |
| Na <sub>2</sub> O              | 1.14   | 1.03   | K              | 1000     |
| H <sub>2</sub> O <sup>-</sup>  | 0.81   | 0.52   | Ti             | 50       |
| Li <sub>2</sub> O              | 6.11   | 5.80   | Cu             | 150      |
|                                |        |        | Be             | 50       |
| Total                          | 100.13 | 100.60 | B              | 50       |

A—Hiddenite from Kabbur Pegmatite, Mysore. Analyst: S. K. Babu.

B—Purple-grey to greenish spodumene from Oorigum, K.G.F., Analyst: E. R. Thirumalachar.

has a slight excess of lithia in comparison to the one reported in literature.

The trace elements justifies the contention of Gabriel *et al.*<sup>5</sup>, "that the impurities exist as isomorphous replacements in the spodumene structure". Further, the presence of 1200 (ppm) of chromium probably explains the emerald-green colour of the hiddenite as well as its pale pleochroism.

The various studies carried out on the mineral points to the mineral to be a monoclinic pyroxene (spodumene) and further the emerald-green colour assigns to the hiddenite variety.

The author places on record his sincere thanks to Dr. R. Krishnan of the Metallurgy Division of the Bhabha Atomic Energy Research Centre for furnishing the X-ray data, and to the Heads of the

Divisions of Health Physics, Spectroscopy and Analytical Chemistry of BARC, for affording laboratory facilities to carry out the chemical and trace element analysis.

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### RECORD OF A SPECIMEN OF MYSTUS MONTANUS (JERDON) WITH STUNTED PECTORAL SPINE

ASYMMETRICAL occurrence of paired organs in bilaterally symmetrical teleosts is rare. Instances of the loss of both pelvic fins such as in *Channa scopoli*, repression of the one pair of mandibular barbels as in *Silonia Swainson* (Majumdar<sup>1</sup>) are on record. However, dissimilarity in the growth of paired fins is not known. A specimen of *Mystus montanus* (Jerdon) 64 mm in length collected from the five falls forest, Courtallam, by the author, on 16-11-1973, has the right pectoral spine dissimilar in length, girth, nature of serration as compared to its counterpart on the left side. The specimen under report was collected from a rocky bottom, in a swift flowing stream of the river Courtallam at Courtallam, along with seven other normal samples of the same species, ranging in standard length from 45 to 85 mm. The spine does not appear to be of a secondary growth, nor injured. Compared to its counterpart on the left side, it is only 8 mm in length *versus* 12 mm of the left side with 4 antrorse teeth (*versus* 8). The serrations are feeble. Some data obtained on the two spines of the fish are as below :

|  |    |         |
|--|----|---------|
| Standard length of the fish                  | .. | 64 mm   |
| Head length                                  | .. | 16 mm   |
| Head length/left pectoral spine<br>(stunted) | .. | 1.06 mm |
| Head length/right pectoral spine             | .. | 1.14 mm |
| The number of pectoral fin rays :            |    |         |
| Right side                                   | .. | 1 + 8   |
| Left side                                    | .. | 1 + 6   |

The spine on the left side which is stunted is 4 mm shorter in length than its counterpart on the

right side, but the diameter is more or less same. The pectoral fin itself is normal.

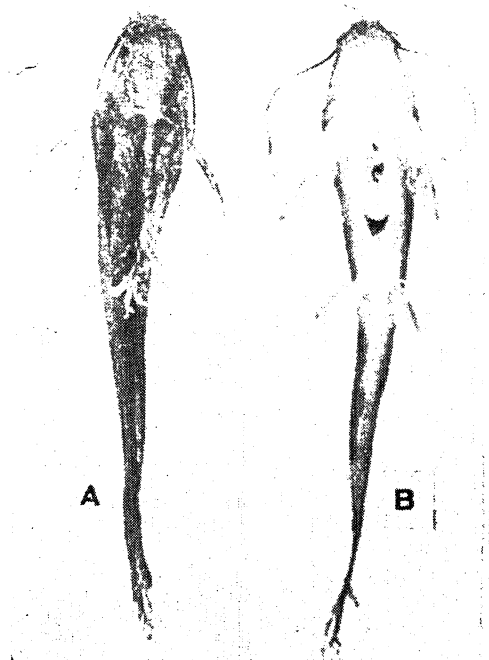


FIG. 1

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# **BIFURCATED BARBEL IN A CATFISH *HETEROPNEUSTES FOSSILIS* (BLOCH)**

REPORTS on the abnormality of different organs in fishes have been made by many authors but literature on the presence of forked barbels in fishes are very few. Tandon and Sharma (1971)<sup>1</sup> and Ovais (1974)<sup>2</sup> have reported the presence of forked barbels in *Callichrous macropthalmus* and *Clarias batrachus* respectively. In the present communication a case of bifurcated barbel in *Heteropneustes fossilis* (Bloch) has been reported which appears to be the first report on the said species.

A female specimen of *H. fossilis*, exhibiting the abnormality, was collected from the local market at Barrackpore. The left innermost maxillary barbel of the specimen was observed to be bifurcated (Fig. 1). The length of the left branch of the

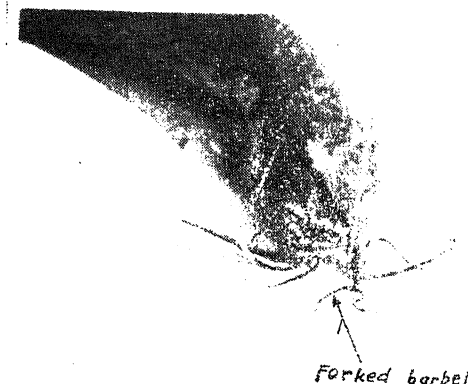


FIG. 1

abnormal barbel from the point of bifurcation was 1.7 cm and that of the right branch was 1.6 cm. The total length of the abnormal barbel was 2.8 cm up to the tip of its left branch and 2.7 cm up to the tip of its right branch. The unbranched corresponding normal right barbel was 2.8 cm. The total length/weight of the specimen was 15.8 cm/14.9 gm.

The cause of such abnormality could not be determined. Tandon and Sharma (1971)<sup>1</sup> were unable to produce forked barbed in experimental induced regeneration of barbels in *H. fossilis*. The abnormality in the fish described here may be due to mechanical injury of the barbel during its early development.

The authors are indebted to Dr. V. G. Jhingran, Director, for his encouragement and to Dr. P. V. Dehadrai, Project Co-ordinator, Air-breathing fish Culture and to Shri B. N. Saigal for helpful suggestions.

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## SOME NEW MARKET DISEASES OF VEGETABLES

DURING recent years much attention has been paid to the post-harvest diseases of vegetables<sup>1-5</sup>. They not only inflict enormous losses to plant products during marketing and storage but also serve as sources of infection through seeds and other plant products. In the present paper, some of the new and interesting diseases of vegetables collected during a survey of the local vegetable markets are described. Symptoms of various diseases were recorded and the colours are recorded as per Ridgway's<sup>2</sup> color chart. The pathogen associated with the disease was isolated on Asthana and Hawker's medium 'A' and was identified. Pathogenicity of the organism was confirmed by inoculating the pathogens on healthy vegetables employing Granger and Horne's<sup>1</sup> method and the diseases have been described hostwise.

*Lagenaria vulgaris* (Dutch) Rusby. (Bottle gourd)

The infection started mostly from the blossom end of the fruit in the form of white patches which later on changed their colour to tawny olive. Some of the patches coalesced and the region beneath became soft. The deeper tissues of the fruit also showed the presence of the fungus. Later on, the entire fruit was affected and as a result, the inner portion of the fruit was completely destroyed. The fungus<sup>6</sup> responsible for the disease was found to be an isolate of *Aspergillus niger* Van Tiegh.

*Luffa cylindrica* Roem. (Sponge gourd)

A severe rot of this fruit was observed during the months of August and September 1973. The infection started either from the apical or basal end of the fruit as olive brown coloured necrotic areas. Some of the necrotic lesions coalesced and infected portion became pulpy. An isolate<sup>6</sup> of *Aspergillus niger* Van Tiegh. was found associated with the diseased fruits.

*Solanum melongena* L. (Brinjal)

The disease appeared in the form of verona brown coloured necrotic areas. These areas enlarged gradually and ultimately occupied major portion of the fruit. The rotted tissue produced a juice emitting foul odour. The disease was more common on deep purple variety than on green variety. Isolations<sup>6</sup> from the diseased portion yielded an isolate of *Aspergillus niger* Van Tiegh.

*Trichosanthes dioica* Roxb. (Parval)

The disease could start at any place on the fruit as ochraceous buff colour spots. The spots enlarged and their colour changed to tawny olive. Sometimes 2 or 3 spots coalesced and formed a bigger spot. On some of the older spots, black fruiting bodies were visible. Isolations<sup>3</sup> from the diseased portion yielded an isolate of *Phoma pomorum* Thum.

*Zingiber officinalis* Rose (Ginger)

The disease started as ochraceous buff to tawny olive coloured irregular depressed areas on the surface of rhizome. These areas, later on, increased in size and occupied major portion of the rhizome. The internal tissue of the rhizome developed a dry rot. In the case of severe infection, the rhizome became smaller in size and lighter in weight. An isolate of *Rhizopus oryzae* Went. and Prinsen was found responsible for the disease. The morphological characters of the isolate were similar to those described by Yamamoto<sup>7</sup>.

Authors are grateful to Prof. D. D. Pant for providing laboratory facilities and to Dr. A. Johnston, Director, C.M.I., Kew, for his help in confirming the identity of pathogens.

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## A RAPID METHOD FOR DETERMINATION OF OSMOTIC POTENTIAL OF PLANT CELL-SAP

IN the study of plant-water relationships assessment of water potential and osmotic potential are important, particularly in plants grown under saline stress, where osmotic potential determines the plant growth<sup>1-4,7</sup>. There are two major methods to determine osmotic potential (osmotic pressure,  $\pi$ ) of plant cell-sap: (a) freezing point depression and vapour pressure method and (b) plasmolysis<sup>1-3</sup>. Both these methods are accurate but rather difficult and time-consuming.

The United States Salinity Laboratory observed that the OP (atm.) of the soil solution is approximately equal to 36% of the electrical conductivity measured in millimhos/cm<sup>6</sup>. Furthermore, the conductivity of soil or plant extract gives a measure of the quantity of salt present. As such it is apparent that moisture content of plant tissue should also be considered. At a given temperature, the OP of a dilute solution is directly proportional to its solute concentration<sup>5</sup>. The OP of non-electro-

lytes is normally very much lower than that of electrolytes. Hence, in the present investigations the use of conductance measurements for the determination of OP has been extended to plant samples. The relation  $OP = 0.36 \times EC \times 10^3 \times$  dilution factor is used in the case of *Rhoeo discolor* leaves, and compared with the standard plasmolytic method. The details are as follows:

One g of fresh leaves of *Rhoeo discolor*, Hance, was ground to a paste in a porcelain mortar, strained through a muslin cloth and made upto 25 ml with distilled water. The electrical conductance of the expressed cell-sap was measured in a 'Elico' conductivity bridge. Moisture content of *Rhoeo* leaves was measured following standard methods of plant analysis. The OP in bars of *Rhoeo* cell-sap was then calculated as:  $=(EC \times 0.36 \times \text{d.f.})/0.987$ , where, EC = electrical conductance in millimhos/cm at 25°C of plant extract; d.f. = dilution factor depending upon the moisture content of the tissue and extract volume:  $0.987 =$  factor for converting atmospheric pressure to bars.

For comparative purposes, OP of *Rhoeo* cell-sap was also determined by standard plasmolytic method, using sucrose as a plasmolytic agent<sup>1,2</sup>. Ten samples were analysed by each method. The results are presented in Table I.

TABLE I  
Osmotic potential of *Rhoeo* cell-sap in bars\*

|   | New method | Plasmolytic method | 'r'    |
|---|------------|--------------------|--------|
| After accounting for moisture in tissue | -7.05      | -7.32              | 0.91** |

\* Mean of ten samples.  
r = Correlation coefficient.  
\*\* Significant at 0.01 P.

TABLE II  
Osmotic potential of sunflower leaves†

| Salinity gm salt/kg of soil (NaCl+CaCl <sub>2</sub> 4:1) | OP in bars | % decrease over control |
|--|------------|-------------------------|
| 0  | -6.13      | ..                      |
| 1.5  | -6.44      | 5.2                     |
| 3.0  | -8.74      | 42.6                    |
| 4.5  | -10.61     | 73.1                    |

† Average of duplicate samples.

The OP, obtained after accounting for moisture (94.3%) in the tissue by the new method, was slightly lower than that obtained by plasmolytic method (Table I). However, there was a highly significant correlation between the two methods.

Applying the new method, the OP was determined using the leaves of a twenty day old sunflower seedlings (*Helianthus annuus* L. var. Sunrise) grown under three salinity levels. The data in Table II indicate decreased OP of plant cell-sap with increase in salinity level. These findings are in conformity with the views expressed by Kramer<sup>4</sup> and Slatyer<sup>7</sup>.

The proposed new method will be of immense use when large samples are to be investigated for the OP of the plant cell-sap.

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#### IN VIVO PRODUCTION OF TRANSELMINASE BY *HELMINTHOSPORIUM APATTARNAE* (DESH. & DESH.)

SINCE Albersheim *et al.*<sup>1</sup> and Nagel and Vaughn<sup>2</sup> reported the non-hydrolytic split of 1,4-glucosidic bonds in pectic substances, many workers tried to explain the role of transeiminative enzymes in the disease development by demonstrating their *in vitro* production by various plant pathogens. But little is known about *in vivo* production of these enzymes. Therefore, an attempt has been made to detect the transeiminase (TE) in the rotted potato tuber caused by *H. apattarnae*.



Fresh and healthy potato tubers were brought from market. They were surface sterilized with rectified spirit in the inoculation room already sterilized with ultra-violet light. After sterilization, 2" deep cavities were made into each tuber with the help of sterile cork-borer diameter 8.0 mm. Five drops of spore suspension prepared from six day old slope culture of *H. apattarnae* were placed in each cavity and potato cylinders were replaced. Potato tubers were inoculated similarly with sterile distilled water and used as control. Tubers were incubated for 7 days at  $25 \pm 1^\circ \text{C}$ . All tubers were placed in the sterilized tin box to avoid contamination. After incubation period, tubers were cut transversely to scrap the rotten portion and its weight was recorded. It was later crushed in mortar with pestle by adding distilled water in 500:1 (mg/ml). The extract was filtered with fine cloth and then centrifuged at 5,000 rpm for 10 minutes to make it cell free. The pH of the supernatant fluid was noted by a pH meter and it was then used as enzyme solution and enzyme activity was determined spectrophotometrically at pH 6.7 and 9.7 by using the procedure of Ayers *et al.*<sup>3</sup>.

TABLE I

*In vivo* detection of transeliminase activity at pH 6.7 and 9.7

| Wave-length (m $\mu$ ) | Absorbance |        |                |        |
|------------------------|------------|--------|----------------|--------|
|                        | Pectin     |        | Na-polypectate |        |
|                        | 6.7        | 9.7    | 6.7            | 9.7    |
| 200                    | ..         | ..     | ..             | ..     |
| 210                    | ..         | ..     | ..             | ..     |
| 220                    | ..         | ..     | ..             | 0.068  |
| 222                    | ..         | ..     | ..             | 0.073  |
| 224                    | ..         | 0.041  | 0.0706         | 0.0969 |
| 226                    | ..         | 0.0655 | 0.0706         | 0.0782 |
| 228                    | ..         | 0.1024 | 0.0706         | 0.0757 |
| 230                    | 0.0996     | 0.1107 | 0.073          | 0.0809 |
| 232                    | 0.1308     | 0.1427 | 0.0756         | 0.073  |
| 234                    | 0.127      | 0.1612 | 0.073          | 0.0757 |
| 236                    | 0.1107     | 0.1135 | 0.073          | 0.0706 |
| 238                    | 0.1051     | 0.0969 | 0.0706         | 0.0706 |
| 240                    | 0.015      | 0.0942 | 0.068          | 0.063  |

Activity of TE was absent in the extract of healthy potato tuber. Extract of potato tuber infected by *H. apattarnae* had shown activity. The TE activity was maximum at pH 9.7 in both the substrates. It was more at pH 9.7 in pectin than in sodium-polypectate solution. Absorption was maximum at 234 m $\mu$  in pectin and at 224 m $\mu$  wavelengths in sodium-polypectate respectively.

*In vivo* detection of these enzymes have been reported by various workers in the case of different fungi. But as regards the activity of these enzymes, different results are obtained for *Fusarium oxysporum* and *F. solani*, Papavizas and Ayers<sup>3</sup>, and *Alternaria compacta* Punde<sup>6</sup>. On the other hand, Heath and Wood<sup>2</sup> could not get polygalacturonate transeliminase activity in the extracts of *Myrothecium pinodes* affected lesions. Studies on *in vivo* production of transeliminases by *H. apattarnae* clearly indicated that the fungus secretes enzymes which are capable of degrading pectic substances transeliminatively. It can be concluded that transeliminases also appear to play an important role in disease development.

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#### EFFECT OF GAMMA IRRADIATION OF SEEDS ON SOME MORPHOLOGICAL CHARACTERS AND SEX EXPRESSION IN MUSKMELON (*CUCUMIS MELO* L.)

SEX expression may be affected by a number of agencies such as light, temperature, mineral nutrition and growth regulators in plants<sup>1</sup>. There are a few reports regarding the effect of gamma irradiation on sex expression in plants<sup>2,3</sup>. The present investigations were undertaken to elucidate the effect of gamma irradiation of seeds on length of vine, nodes, lateral branches, sex expression and pollen sterility in *C. melo* L.

The investigations were carried out from the middle of February to middle of June, 1973. Healthy seeds were dried in the sun for five days (10 a.m. to 4 p.m., 26–30° C). The seeds were

TABLE I

Effect of gamma irradiation of seeds on morphological characters and sex expression in *Cucumis melo* L.

(Values are mean with  $\pm$  S.E. of mean)

| Observations                                   | Gamma irradiation (kR) |                 |                 |                  |                  |                  |
|--|------------------------|-----------------|-----------------|------------------|------------------|------------------|
|  | Control                | 0.5             | 1.0             | 1.5              | 2.0              | 2.5              |
| Length of vine (in m)                          | 1.8 $\pm$ 0.051        | 2.1 $\pm$ 0.067 | 3.2 $\pm$ 0.092 | 2.4 $\pm$ 0.070  | 1.5 $\pm$ 0.42   | 1.3 $\pm$ 0.054  |
| Nodes  | 31 $\pm$ 0.91          | 34 $\pm$ 0.87   | 33 $\pm$ 0.70   | 28 $\pm$ 0.54    | 18 $\pm$ 0.47    | 16 $\pm$ 0.33    |
| Lateral branches                               | 8 $\pm$ 0.11           | 9 $\pm$ 0.20*   | 11 $\pm$ 0.16   | 10 $\pm$ 0.22    | 7 $\pm$ 0.18     | 2 $\pm$ 0.05     |
| No. of node bearing first staminate flower     | 6.3 $\pm$ 0.16         | 6.5 $\pm$ 0.16† | 7.0 $\pm$ 0.11* | 8.2 $\pm$ 0.27   | 8.6 $\pm$ 0.18   | 11.7 $\pm$ 0.28  |
| No. of node bearing first hermaphrodite flower | 14.9 $\pm$ 0.30        | 12.2 $\pm$ 0.21 | 10.5 $\pm$ 0.37 | 15.0 $\pm$ 0.20† | 15.3 $\pm$ 0.31† | 15.1 $\pm$ 0.25† |
| Staminate flowers                              | 205 $\pm$ 6.1          | 196 $\pm$ 5.7   | 187 $\pm$ 5.2   | 171 $\pm$ 4.9    | 161 $\pm$ 4.6    | 109 $\pm$ 3.2    |
| Hermaphrodite flowers                          | 10 $\pm$ 0.16          | 16 $\pm$ 0.28   | 20 $\pm$ 0.54   | 12 $\pm$ 0.18    | 9 $\pm$ 0.11†    | 5 $\pm$ 0.08     |
| Ratio of staminate/hermaphrodite flower        | 20.5 : 1               | 12.2 : 1        | 9.3 : 1         | 14.2 : 1         | 16.2 : 1         | 21.2 : 1         |
| Pollen sterility % of staminate flowers        | 12                     | 34              | 62              | 67               | 73               | 81               |
| Pollen sterility % of hermaphrodite            | 72                     | 78              | 80              | 86               | 88               | 97               |

\* Significant at 5% level.

† Not significant.

irradiated with five doses, viz., 0.5, 1.0, 1.5, 2.0 and 2.5 kR from Co<sup>60</sup> source of gamma rays at a dose rate of 2100 R/minute in air. Two hundred seeds were irradiated for each dose. Seeds of *C. melo* cv Hara Madhu were sown on the one side of 30 m long and 8 m wide plots. Three replicates of ten plants were taken for each dose. Pollen sterility % was isolated by germinating pollen in 10% sucrose and 3% agar medium.

Irradiation of seeds increased the length of vines, the number of nodes and lateral branches upto 1 kR and with higher doses these factors decreased (Table I). The first staminate flower appeared on higher nodes while the first hermaphrodite flower appeared on lower nodes up to 1 kR. Irradiation with higher doses did not affect the position of first hermaphrodite flower but increased the node number to the first staminate flower. Irradiation upto 1.5 kR increased the number of hermaphrodite flowers, 2.0 kR had no significant effect and 2.5 kR reduced it drastically. Number of staminate flowers decreased progressively with increase in dose. Irradiation caused a shift towards hermaphrodite flowers. Pollen sterility increased gradually with increase in dose (Table I).

Our findings suggest that 1.0 kR irradiation is an effective dose for inducing better vegetative growth. Similar results have also been reported for *Cucumis sativus*<sup>2</sup> and *Lycopersicum esculentum*<sup>5</sup>. The irradiation of seeds suppresses maleness and promotes femaleness. This clearly shows that irradiation has properties opposite to gibberellins. Gibberellins are known to induce maleness<sup>4,7</sup>. Recently it was found that irradiation induced ethylene production<sup>6</sup>. It is, therefore, possible that irradiation exerts its effect on sex expression through ethylene evolution system. Ethylene has also been reported to cause femaleness<sup>7</sup>.

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### COLCHICINE INDUCED AMPHIDIPLOID BETWEEN MUNG (*PHASEOLUS AUREUS* ROXB.) AND URD (*PHASEOLUS MUNGO* L.)

INTERSPECIFIC hybridization is resorted to when the desired character is not found within the species. But in most of the cases, the fertility of species hybrids has been found to be very poor. The fertility of such hybrids may be restored through chromosomal doubling. Colchicine treatment is one of the efficient techniques of doubling the chromosomes. Sen and Ghosh<sup>1</sup> and Dana<sup>2</sup> attempted the cross between mung (*Phaseolus aureus* Roxb.) and urd (*Phaseolus mungo* L.) and reported that the fertility of  $F_1$  was very low. The present investigation was undertaken to overcome the problem of fertility in the aforesaid species hybrid by the colchicine treatment.

parent. Three  $F_1$  seedlings were treated each with 0.25, 0.50, 1.00 and 1.50% aqueous solution of colchicine at two leaf stage during *kharif* 1972. The reagent was applied at the shoot apex for half an hour daily for seven days. Observations were recorded and polyploid plants were identified. During *kharif* 1973 amphidiploid  $F_1$  and the parents were grown in a completely randomised design with 5 replications.

#### Results and Discussion

Three seedlings were treated each with 0.25, 0.50, 1.00 and 1.50% aqueous solution of colchicine. One colchipsoid plant was obtained with 0.25% and three plants with 0.50% concentration. Polyploidy could not be induced with 1.00 and 1.50% colchicine.

The colchicine treatment resulted in the inhibition of seedling growth. The seedlings showed abnormalities like swelling of stem apex, thickening of stem and leaves and broadening of leaflet and flower size, etc. The seedlings which were treated with 1.00 and 1.50% colchicine died after some time.

The amphidiploid differed significantly from the  $F_1$  in respect of length and width of leaflet, stipule and flower (standard); number of cluster/plant; number of pod per cluster; pod length; number of seed per pod and 1000 grain weight (Table I).

TABLE I

Comparison of characters of amphidiploid,  $F_1$  and parents (mung T.44 and urd T.9)

| Character                      | Amphidiploid | $F_1$ | Parents |       | C.D. at 5% |
|--------------------------------|--------------|-------|---------|-------|------------|
|                                |              |       | T. 44   | T. 9  |            |
| Plant height (cm)              | 42.30        | 43.10 | 36.70   | 40.30 | 3.567      |
| No. of primary branches        | 2.90         | 2.70  | 2.10    | 3.70  | 0.640      |
| Length of leaflet (cm)         | 9.99         | 8.14  | 8.73    | 8.61  | 0.711      |
| Width of leaflet (cm)          | 9.34         | 7.51  | 7.39    | 5.40  | 0.584      |
| Length of stipule (mm)         | 16.10        | 13.20 | 10.90   | 12.20 | 0.784      |
| Width of stipule (mm)          | 5.30         | 4.20  | 5.20    | 3.50  | 0.329      |
| Length of flower standard (mm) | 22.30        | 17.00 | 16.70   | 16.60 | 0.683      |
| Width of flower standard (mm)  | 17.10        | 14.10 | 13.70   | 12.60 | 0.992      |
| Days to maturity               | 86.50        | 88.60 | 64.10   | 84.80 | 4.779      |
| No. of cluster per plant       | 8.40         | Nil   | 5.10    | 10.40 | 1.720      |
| No. of pod per cluster         | 6.10         | 0.70  | 5.20    | 5.10  | 0.905      |
| No. of seed per pod            | 4.70         | 2.10  | 10.90   | 5.90  | 0.255      |
| Pod length (cm)                | 4.60         | 4.10  | 7.16    | 4.10  | 0.404      |
| 1,000 seed weight (g)          | 44.60        | ..    | 35.70   | 40.00 | 1.493      |

#### Material and Methods

Crosses between mung T. 44 and Urd T. 9 were made during summer 1972 using the former as female

The expression of plant height, number of primary branches and period of maturity did not differ from  $F_1$ .



favouring optimal lesion production are, perhaps hitherto, unknown.

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#### UTILIZATION OF SULPHUR COMPOUNDS BY *ALTERNARIA TRITICINA*

PRASADA AND PRABHU<sup>2</sup> reported a leaf blight disease of wheat and identified the pathogen as *Alternaria triticina* Prasada and Prabhu. The disease has assumed epidemic proportions in India, with the introduction of high yielding varieties having 'durum' blood. A detailed investigation of nutritional requirements and host pathogen relationships of this pathogen has been taken up in this laboratory and the effect of different sulphur compounds on the growth of *Alternaria triticina* is presented here.

Czapek-Dox liquid medium with 3% sucrose was used as the basal medium. Different sulphur compounds were added to the medium in quantities which supplied the same amount of sulphur present in 0.5 g of  $MgSO_4 \cdot 7H_2O$ . The pH of the medium was adjusted to 5.0 before sterilization. The flasks containing 25 ml of the medium were seeded with 1 ml aliquots of uniform spore suspension. The cultures were incubated at room temperature (25–30° C) in dark and the growth of the fungus was determined as dry weight of mycelium after 10 days. For each treatment four replicates were maintained. The pH values of the culture filtrates were also recorded.

From the data (Table I) it is evident that *A. triticina* shows significant differences in the utilization of different sources of sulphur. Magnesium sulphate supported maximum growth. The same effect was reported by Hasija<sup>1</sup> for *A. citri* and *tenuis* and by Singh and Khanna<sup>4</sup> for *A. tenuis*. The latter fungus is frequently associated with blighted wheat leaves as a saprophyte<sup>3</sup>. Sodium sulphate, sodium thio-sulphate, sodium bisulphate, potassium bisulphate, sodium sulphite, potassium thiocyanate, ammonium sulphate and potassium persulphate supported progressively less growth of the fungus. There was no growth on potassium metabisulphite and growth was very poor on thiourea which may be, as

TABLE I  
Effect of different sulphur compounds on the  
growth of *Alternaria triticina*

| Sulphur source           | Dry wt. of<br>mycelium<br>in mg<br>(Average of<br>4 replicates) | pH values of<br>culture<br>filtrates |
|--------------------------|---|--------------------------------------|
| Ammonium sulphate        | 107   | 6.3                                  |
| Magnesium sulphate       | 237   | 8.0                                  |
| Potassium bisulphate     | 144   | 7.0                                  |
| Potassium metabisulphate | Nil   | 5.0                                  |
| Potassium persulphate    | 106   | 6.5                                  |
| Potassium thiocyanate    | 120   | 6.0                                  |
| Sodium bisulphate        | 156   | 6.8                                  |
| Sodium sulphate          | 165   | 7.6                                  |
| Sodium sulphite          | 131   | 7.2                                  |
| Sodium thiosulphate      | 160   | 6.6                                  |
| Thiourea                 | 15  | 5.5                                  |
| No sulphur               | 89  | 5.7                                  |

F. test—Replicates : Insignificant  
Treatments : Significant  
S.E. : 4.7  
C.D. at 5% : 13.34.

reported by Lily and Barnett<sup>2</sup>, due to the inability of the pathogen to break the carbon sulphur bond. Generally there is an increase in the pH values of the culture filtrates (5.5–8.0) where there was growth. The results indicate that *A. triticina* is capable of utilizing a wide variety of sulphur compounds but the growth response is better on sulphates.

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## EFFECT OF ETHREL ON ASCORBIC ACID OF COFFEE FRUITS

ETHREL spray on plants releases ethylene directly to plant tissues producing numerous physiological effects and regulate plant development, out of which hastening of fruit ripening is one<sup>1</sup>. As in other fruits, in coffee also the natural ripening agent is ethylene<sup>2</sup>. After spraying ethrel on mature green coffee fruits, it enters into fruit cells, releases ethylene and hastens the ripening process<sup>3</sup>. Since quantitative changes of ascorbic acid are depended mainly on carbohydrate metabolism<sup>4</sup>, changes in its content may occur with ethrel spray which was found to influence the carbohydrate metabolism in ripe coffee fruits (Authors' unpublished work).

During 1973 crop season, nine plants of *Coffea arabica* L. cv. S. 795 at random were sprayed each with 750 ml aqueous solution containing 0.5 ml ethrel (2-chloroethane phosphonic acid, an Amchem product, Ambler, U.S.A.). The plants (19 years old) were grown under natural shade at Central Coffee Research Institute. The fruits were sprayed when they were in mature green condition. Thirteen days after spray, fully ripe fruits were collected from sprayed plants and also from nine control (unsprayed) plants at random from the same plot. Fruit wall, mucilage and seed were separated and their ascorbic acid was determined<sup>5</sup>.

The ascorbic acid was significantly (at 1%) lower in fruit wall with ethrel spray as compared to that of control, whereas in mucilage it was significantly (at 1%) more with ethrel treatment than of control (Table I). Even though the vitamin content was more in seeds by about 9% with ethrel spray as compared to control, the increase was not statistically significant.

TABLE I

Effect of ethrel spray on ascorbic acid (mg/100 g fresh weight) in ripe fruits of *arabica* coffee cv. S. 795

| Fruit component | Control (unsprayed) | Ethrel sprayed | C.D. at |      |
|-----------------|---------------------|----------------|---------|------|
|                 |                     |                | 5%      | 1%   |
| Fruit wall      | 6.02                | 1.72           | 3.77    | NS   |
| Mucilage        | 0.58                | 1.11           | 0.30    | 0.50 |
| Seed            | 45.15               | 49.02          | NS      | NS   |
| Total           | 51.75               | 51.85          |         |      |

NS: Not significant.

Distribution of ascorbic acid in different components of green and ripe fruits of five coffee types belonging to three coffee species, and of pulp and

pulped water of bulk arabica has been reported<sup>6-8</sup>. However, ethrel spray has not caused any adverse effect on ascorbic acid content in the fruits as a whole, except for the quantitative changes in different fruit components as compared to the fruits of control (unsprayed) plants.

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## TWO NEW DISEASES OF SAFFLOWER FROM INDIA

SAFFLOWER (*Carthamus tinctorius* Linn.) is an important oil seed crop cultivated in several States of India. During survey in Varanasi and Mirzapur Districts, two new root diseases were observed.

### 1. Wilt of Safflower

The disease appears at different growth stages. A characteristic symptom is unilateral yellowing of the foliage followed by wilting (Fig. 1). Half of the leaf gets discoloured with the midrib usually curved towards the chlorotic side. Some plants look stunted coupled with dark green foliage and with distorted shape. Yellowing starts from the lower leaves and progresses upward. The affected leaf tissue generally turns brown and gets killed. Dark brown discolouration in the vascular tissues can be traced from the root to the upper part of the stem (Fig. 2). In a few cases creamy white mycelium was observed at collar region progressing externally upward on the stem.

The pathogen was isolated on P.D.A. from diseased roots. Mycelium delicate, white or peach usually with a purple tinge, sparse to abundant then floccose becoming felted and sometimes wrinkled in old cultures. Microconidia borne on simple phialids arising laterally on the hypha or on short sparsely branched conidiophores, abundant, oval-ellipsoid cylindrical, straight to curved,  $5-12 \times 2.2-3.5 \mu$ . Macroconidia sparse borne on more elaborately branched conidiophores. They are thin walled, generally 3-5 septate, fusoid-subulate and pointed at both ends. Three septate spores in range  $27-46 \times 3-4.5 \mu$  is most common. Chlamydospores smooth walled, generally abundant and are both terminal and intercalary, usually solitary but occasionally formed in pairs.

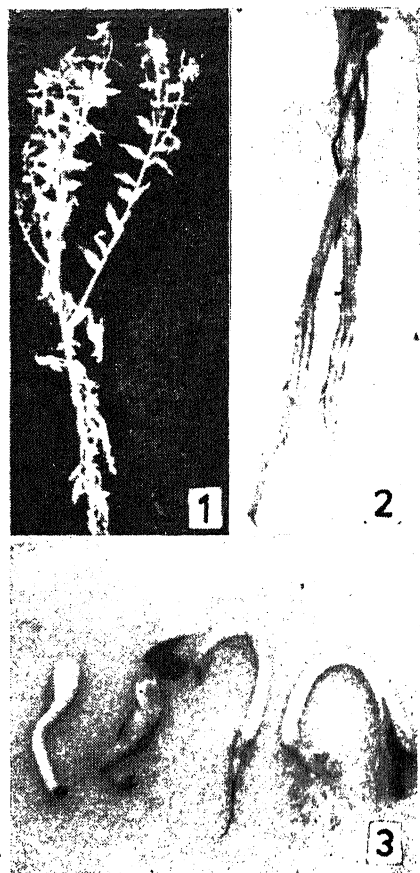
The fungus has been identified as *Fusarium oxysporum* f. sp., *carthami* and has been confirmed by C.M.I. (IMI-166917). Pathogenicity test was proved following the method suggested by Klisiewicz and Thomas (1970).

The disease was first reported from California (Klisiewicz and Houston, 1962) and this is the first report of its occurrence in India. The disease incidence was estimated at 5-15% in most of the fields but in some it was as high as 25%.

## 2. Damping-off of Safflower

The disease was observed at a very early stage. In the morning hours affected plants droop and topple over either individually or in patches. The emerging radicle gets infection and soft lesion formed which progresses upward causing death of the seedling (Fig. 3). The fungus was isolated from diseased seedlings on P.D.A. Cultures at first white with floccose aerial mycelium tinged with peach but after 7-14 days changed to beige and finally to deep olive buff. From below initially peach coloured changing to vinaceous fawn and finally dark brown. Conidia at first sparse and produced on simple lateral phialids,  $10-12.5 \times 2.5-3 \mu$ , on aerial mycelium. After about 14 days conidia are more abundant with the production of compact penicillately branched conidiophores. These arise from a lateral branch which initially may be of one cell and bears 2-4 phialids at the apex, or 2-4 branches may form at the apex, each of which may produce several phialids. These phialids are generally obclavate,  $12-17 \times 3-4 \mu$ . Sporodochia generally absent. The conidia are falcate with a well developed pedicellate foot cell and an attenuated apical cell which is bent inwards exaggerating the normal curve of the spore. Mature conidia have 4-7 thin but distinct septa measuring  $22-60 \times 3.5-5.9 \mu$ . Chlamydospores intercalary, solitary, in chains or in knots, globose,  $7-9 \mu$  in diameter. Intercalary

chlamydospores in the mycelium are formed singly or in chains. Perithecia not observed.



FIGS. 1-3. Fig. 1. Wilt of safflower incited by *Fusarium oxysporum* f. sp. *carthami* showing unilateral wilting. Fig. 2. Vascular discoloration of safflower roots due to *F. oxysporum* f. sp. *carthami*. Fig. 3. Damping off of safflower seedlings incited by *F. equiseti*.

The fungus has been identified as *Fusarium equiseti* (Corda) Sacc. and confirmed by C.M.I. (IMI-166921). Pathogenicity test was proved following method suggested by Klisiewicz and Thomas (1970). Damping-off of safflower incited by *F. equiseti* is a new disease reported for the first time.

Cross inoculation tests on young seedlings and 21 day old plants of safflower were carried out and invariably negative results were obtained. Thus confirming that the above two diseases are distinct and are incited by two different organisms.

Thanks are due to Dr. C. Booth of the Commonwealth Mycological Institute, Kew (U.K.), for confirming the identification. Thanks are also due

to CSIR, New Delhi and UGC for award of fellowships to AKS and DKC respectively.

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# REVERSAL OF CHLORAMPHENICOL INHIBITED GROWTH BY RIBOFLAVIN IN GREEN GRAM (*PHASEOLUS RADIATUS* L.)

UNTIL recently it was felt by many physiologists that no B vitamin is implicated in protein synthesis. The concept that B vitamins act merely as catalysts and coenzymes is becoming more and more fluid. By virtue of possessing a pyridine ring as the principal chemical constituent with nitrogen as an essential element. B group vitamins appear to influence many physiological processes. The present knowledge as to what physiological processes are influenced by B vitamins is very cursory. That nicotinic acid effects nucleic acid and protein synthesis as well as growth and yield was shown by Bogdonova<sup>1</sup>. The present study was designed to understand the mechanism of action of riboflavin (B<sub>2</sub>) in chloramphenicol inhibited growth. Considerable literature is available concerning the protein inhibiting nature of chloramphenicol.

Green gram seeds (cultivar) were subjected to presowing treatment for 24 hr in 20 ppm of chloramphenicol, 20 ppm of riboflavin and their combination in Petri dishes in a luminosity of 1000 Lux and at 25° C ± 1° after which the seed-

lings were transferred to distilled water and allowed to grow for a period of one week. The starch content was estimated according to the method of McCready *et al.*<sup>2</sup>. The activity of amylase was determined by the method of Bernfeld<sup>3</sup>. The growth of the seedlings was measured in terms of fresh and dry weights as well as length of the seedlings.

The fact that chloramphenicol is an inhibitor of protein synthesis was well established<sup>4</sup>. Prior to its action on protein synthesis it appears from the present study that the hydrolysis of starch to reducing sugars is inhibited thus reducing the substrate for respiration consequently affecting growth. The action of chloramphenicol on starch hydrolysis (as evinced by amylase activity) may be preceded by its action on protein synthesis as starch is the first metabolite to be acted upon. This statement can be substantiated by the observation made by Nurten<sup>5</sup> that the primary action of chloramphenicol is in the inhibition of water uptake and water permeability in potato.

Riboflavin treatment caused an increase in starch hydrolysis which is also associated with increase in growth. An earlier report by Gopala Rao<sup>6</sup> indicated that riboflavin increases respiration, protein and chlorophyll content. In the present study it was observed that although starch hydrolysis is high the activity of amylase is low which may be apparently due to an inhibitory action of riboflavin on the enzyme amylase. It is quite possible that other enzymes concerned with starch hydrolysis, *viz.*, starch phosphorylase are activated. It is a well-known fact that enzymes such as R, and Z, P and Q are also involved in starch hydrolysis<sup>7</sup>.

The reversal of chloramphenicol inhibited growth by riboflavin (78% on the fifth day—Table I) supports the earlier observation by Gopala Rao<sup>6</sup>

TABLE I

| Days after sowing | Control | Chloramphenicol | Riboflavin | Chloramphenicol + Riboflavin |
|-------------------|---------|-----------------|------------|------------------------------|
| 1. Growth         | 0.83    | 0.72            | 0.84       | 0.80<br>(11.10)              |
| Starch            | 456     | 481             | 440        | 448                          |
| Amylase activity  | 0.080   | 0.055           | 0.075      | 0.080                        |
| 2. Growth         | 2.94    | 1.91            | 3.54       | 2.54<br>(32.90)              |
| Starch            | 392     | 467             | 376        | 426                          |
| Amylase activity  | 0.175   | 0.105           | 0.150      | 0.135                        |



TABLE I—(Contd.)

| Days after sowing | Control | Chloramphenicol | Riboflavin | Chloramphenicol +<br>Riboflavin |
|-------------------|---------|-----------------|------------|---------------------------------|
| 3. Growth         | 5.37    | 2.92            | 6.18       | 4.06<br>(39.00)                 |
| Starch            | 297     | 411             | 258        | 362                             |
| Amylase activity  | 0.140   | 0.120           | 0.130      | 0.130                           |
| 4. Growth         | 10.87   | 5.57            | 15.19      | 9.65<br>(73.20)                 |
| Starch            | 213     | 393             | 213        | 313                             |
| Amylase activity  | 0.240   | 0.170           | 0.215      | 0.195                           |
| 5. Growth         | 18.15   | 8.90            | 21.22      | 15.84<br>(78.10)                |
| Starch            | 123     | 190             | 114        | 176                             |
| Amylase activity  | 0.390   | 0.310           | 0.355      | 0.385                           |
| 6. Growth         | 21.09   | 12.32           | 24.21      | 18.99<br>(54.10)                |
| Starch            | 77      | 123             | 59         | 86                              |
| Amylase activity  | 0.280   | 0.235           | 0.265      | 0.270                           |
| 7. Growth         | 24.16   | 18.21           | 24.56      | 22.58<br>(23.90)                |
| Starch            | 47      | 53              | 36         | 47                              |
| Amylase activity  | 0.190   | 0.320           | 0.175      | 0.295                           |

Note: The figures in the parenthesis represent percentage of reversal by riboflavin.  
Growth (length in cm) The values are the means of ten replications.

Starch (mg/gm dry wt).

Amylase activity (expressed as optical density/ml of homogenate). } The values are the means of three replications.

that riboflavin activates protein synthesis thus causing an increase in growth of the seedlings (50% over control on the 4th day in the present study). With reference to the interaction of riboflavin and chloramphenicol on amylase activity the values are intermediate between riboflavin and chloramphenicol indicating the capacity of riboflavin to partly nullify the effect of chloramphenicol even with regard to amylase activity.

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#### CHROMOSOME NUMBER OF THE PARAMPHISTOME [*GIGANTOCOTYLE EXPLANATUM* (NASMARK, 1937)]

THE Paramphistome group of digenetic trematodes are important from medical as well as veterinary standpoints in view of their common infestation of animals and humans. Divergences of opinion and ambiguities exist regarding their cytology and

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taxonomy. Reliable descriptions of chromosome numbers, their behaviour and karyotypes of several Paramphistomes have not been available<sup>1-10</sup>. There is also a lack of knowledge on the chromosomes of several unexplored species from these angles. *Gigantocotyle explanatum*<sup>11,12</sup> is one such parasite commonly found in the livers, bile ducts and the gall bladders of domesticated ruminants. The chromosome number of this Paramphistome has been reported here.

The adult parasites were collected from the livers of buffaloes. Out of twenty livers examined fourteen of them showed heavy infection. They were processed for the chromosome analysis by the method described earlier by the authors<sup>13,14</sup>. All the divisional stages were traced to the haematoxylin squashes of testis of forty parasites.

The chromosome number was established as  $2n = 18$  from the counts of fifty well spread spermatogonial metaphases. A representative karyotype is presented in Fig. 1. It consists of five pairs of submetacentric, two pairs of metacentric (pairs 6 and 7) and two pairs of acrocentric (pairs 2 and 9) chromosomes. The number was also confirmed by screening two hundred meiotic stages. The chromosomes seen during diplotene (Fig. 2), very late diakinesis (Fig. 3) and metaphase I (Fig. 4) corroborate the above observation. Basing observations on gametogenesis of *G. bathycotyle* from sectioned material, Willmott<sup>1</sup> described the number as  $2n = 12$  consisting of eight short and four long chromosomes. It was also further opined

that no accurate counts and descriptions were possible. Much of reliance cannot, however, be placed on sections for such critical observations in view of their limitations. The chromosome number of the only other species of the same genus unexplored from this aspect has been established here as  $2n = 18$  along with the classification. This indicates that there is a variation in number at the level of the species. While Short and Menzel<sup>15</sup> contended that only the genera could be distinguished based on chromosome numbers, the results reported here are relevant in that such a distinction could be made not only within a genus but between species.

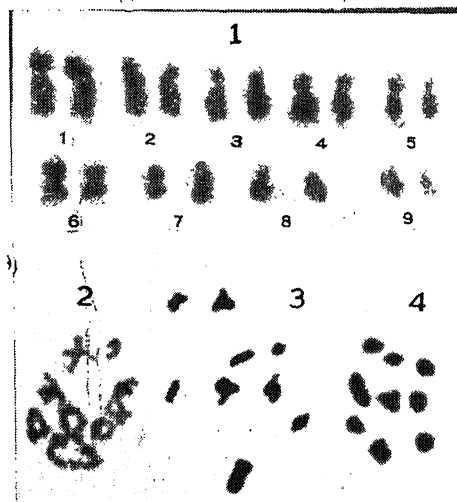
The authors are thankful to Profs. O. S. Reddi and P. Ramachander Rao for their interest and to Dr. T. Vasudev for the identification of the parasite.

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FIGS. 1-4. Fig. 1. Karyotype of *Gigantocotyle explanatum*,  $\times$  ca. 2,500. Figs. 2-4. Diplotene, late diakinesis and metaphase I respectively,  $\times$  ca. 1,300.

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**EXPERIMENTAL INDUCTION OF  
"INTERCOTYLEDONARY INTERNODE" IN  
MATURE EMBRYOS OF *AZADIRACHTA INDICA*  
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RECENTLY it has been shown that the excised embryos of *Azadirachta indica* grown in distilled water or simple mineral solutions exhibit rare morphogenetic responses<sup>1</sup>. The phenomenon included the production of callus followed by bud formation in the plumular region in entire and decapitated seedlings. While conducting surgical experiments on the mature embryos of *A. indica* the present authors noticed another rare morphogenetic expression—the production of an "intercotyledonary internode" between the two cotyledons.

The mature embryos exhibit two cotyledons disposed in an opposite manner. During the present study a majority of the germinating seedlings maintained this condition. However, the embryos subjected to surgical treatment behaved differently.

The embryos were removed from mature fruits which were opened after surface sterilization with 70% alcohol. After soaking them in sterile distilled water for an hour the brown seed coat was removed exposing the cotyledons and the embryonal axis. The hypocotyl apex was removed by a transverse cut with a sterile blade. After this operation the embryo was allowed to grow in sterile petri dishes over cotton wads soaked in Hoagland's mineral solution. While a majority of the treated embryos developed a hypocotyl and adventitious roots and retained the cotyledons in an opposite position, a few were different. The latter showed the displacement of the cotyledons from the opposite position to an alternate one. In this process an internode was formed between the two cotyledons (Fig. 1).

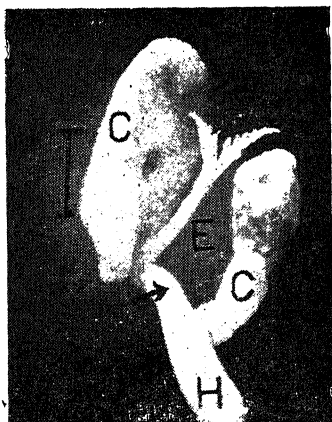


FIG. 1. *Azadirachta indica*. Photograph showing a seedling with intercotyledonary internode (Arrow). Vertical bar is 5 mm. C, Cotyledon; E, Epicotyl; H, Hypocotyl.

It was thought that a re-examination of the alignment of the cotyledons in a large number of embryos may reveal the prevalence of alternate condition in some. A survey indicated that the cotyledons were so closely situated on the embryonal axis that it was difficult to discover incipient 'internodes' between cotyledons. The surgical removal of the hypocotyl apex was repeated and the embryos grown on mineral solutions. It was later discovered that the occurrence of intercotyledonary internodes was frequent in specimens which accidentally received a superficial

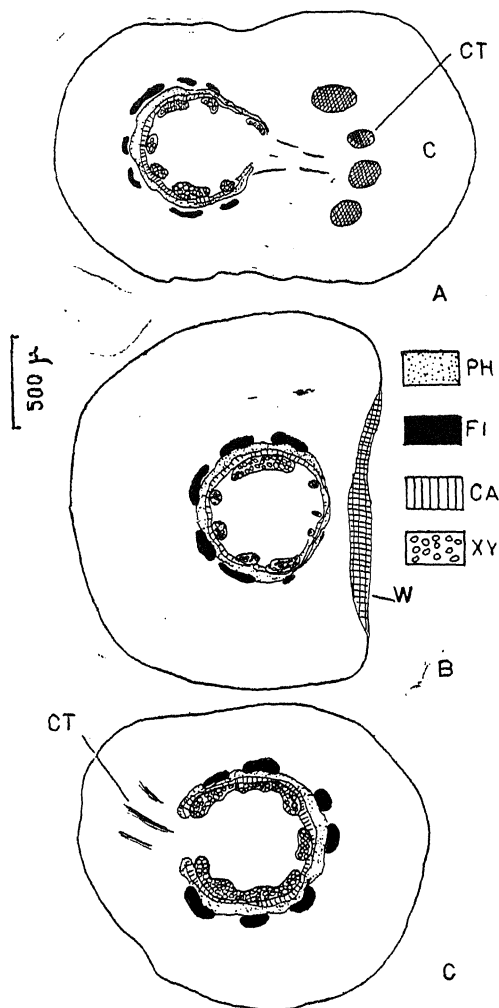


FIG. 2. *Azadirachta indica*. Diagrams showing the disposition of vascular tissues in the seedling shown in Fig. 1. A, Transverse section through the upper cotyledonary node. B, Transverse section through the intercotyledonary internode. C, Transverse section through the lower cotyledonary node. CC, Cotyledon; CA, Cambium; CT, Cotyledonary trace; FI, Phloem fibres; PH, Phloem; XY, Xylem.

surgical injury just below one of the cotyledons. An anatomical study of such embryos (Fig. 2, A-C) indicated that the injury was confined to a side of the axis and did not reach the vascular cylinder. There was initiation of cork cambial activity in the injured region. The elongation of axis lying between the cotyledons resulted in the formation of the 'intercotyledonary internode' and consequent change from opposite to alternate condition of the cotyledons. It was found that the intercotyledonary internode elongated like any normal internode and the distance by which the cotyledons were separated was considerable, being up to 10 mm in 15 days.

The altered nodal condition was studied in transverse sections of the axis (Fig. 2, A-C). It was found that a central double strand and two laterals were given off to each cotyledon. Cambial formation in the intercotyledonary internode was normal. Secondary phloem and xylem were formed. There was normal disposition and differentiation of phloem fibres.

The formation of the intercotyledonary internode indicates that alternate 'phyllotaxy' of the cotyledons may be a possibility. This is likely to alter the emphasis on theories which are based on the opposite condition as a general rule. It is likely that surgical injury in this experimental study induced the incipient internodes to develop fully and result in alternate cotyledons.

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### PLEUROTUS SAJOR-CAJU (FR.) SINGER, A PROTEIN RICH NITROGEN FIXING MUSHROOM FUNGUS

In a search for new sources of protein food to improve the nutritional quality of our diet we came across an edible mushroom in *Pleurotus sajor-caju* (Fr.) Singer which has been reported to produce sporophores in the greenhouse under artificial culture<sup>1</sup>. The fungus has been found to grow readily on farm wastes, producing sporophores and in the process fixing atmospheric nitrogen to enrich the substratum.

The data in support of this are presented in this paper.

Pure cultures of *Pleurotus sajor-caju* obtained from Dr. C. L. Jandaik, Indian Agricultural Research Institute, New Delhi, was used in these studies. Fresh isolates of the fungus were obtained from fresh sporophores, by the tissue culture method and were maintained on oatmeal agar slants. For *in vitro* study the fungus was grown on synthetic media<sup>2</sup>, without nitrogen source. Analar grade chemicals and double distilled water were used to avoid nitrogen in the medium. For spawning beds maize grain spawn<sup>3</sup> was used. A mixture of paddy straw, wheat straw and hulled maize cobs in the ratio of 1 : 1 : 1 (W/W) was used as a substrate (bed) for the production of the mushroom. The substrate was pre-soaked for 12 hr in water and spread in trays (60 × 45 × 15 cm) and spawned with about 500 g of grain spawn. Estimation of protein<sup>4</sup> in the sporophores and the spawned substrate was carried out at periodical intervals.

The growth *in vitro* and protein content of *P. sajor-caju* estimated at periodical intervals are presented in Table I. The fungus not only put forth

TABLE I  
In vitro growth and protein content of *Pleurotus sajor-caju* in nitrogen-free medium

| Incubation period          | Mycelial dry wt. in mg/100 ml | % mycelial nitrogen | % mycelial protein | % N in the liquid medium |
|----------------------------|-------------------------------|---------------------|--------------------|--------------------------|
| 1st week after inoculation | 43                            | 2.1                 | 13.5               | ..                       |
| 2nd week                   | 80                            | 2.28                | 14.3               | ..                       |
| 3rd week                   | 107                           | 2.75                | 17.2               | 0.09                     |
| 4th week                   | 120                           | 2.81                | 17.6               | 0.12                     |
| 5th week                   | 130                           | 2.91                | 18.2               | 0.19                     |

good growth in the nitrogen-free medium but also its nitrogen fixing ability was indicated by increased N-content of the mycelium over a period of five weeks. Nitrogen fixation by higher fungi has been reported by Ginterova<sup>5</sup>.

The nitrogen content of the bed, inoculated with *P. sajor-caju* at different intervals, is presented in Table II. There is a steady increase in the nitrogen content of the substrate upto 30 days of spawning

TABLE II

*Nitrogen content of the bed and yield data of Pleurotus sajor-caju  
(average of four replications)*

| Particulars               | % nitrogen | % protein | Date of<br>harvest<br>of mushroom | Yield in<br>g/bed of<br>3 kg |
|---------------------------|------------|-----------|-----------------------------------|------------------------------|
| Uninoculated bed          | 0.25       | 1.56      | ..                                | ..                           |
| Inoculated bed            | 0.31       | 1.93      | ..                                | ..                           |
| 5 days after inoculation  | 0.97       | 6.06      | ..                                | ..                           |
| 10 days after inoculation | 2.17       | 13.56     | ..                                | ..                           |
| 20 days after inoculation | 2.94       | 18.37     | 23rd day<br>32nd day              | 320<br>120                   |
| 30 days after inoculation | 2.92       | 18.25     | 39th day                          | 100                          |
| 40 days after inoculation | 2.53       | 15.81     | 45th day<br>55th day              | 105<br>250                   |
| 50 days after inoculation | 2.32       | 14.50     | Nil                               | ..                           |
| 60 days after inoculation | 2.23       | 13.93     | Nil                               | ..                           |
| Total mushroom yield      |            |           |                                   | 895                          |

**Protein content of sporophores 34.5%**

|                              |   |          |
|------------------------------|---|----------|
| Total N in the beginning     | : | 7.44 g   |
| N fixed through sporophore   | : | 63.36 g  |
| N fixed in the substratum    | : | 46.08 g  |
| Total N fixed during 60 days | : | 109.44 g |

after which there is a decline. The increased protein content of the substratum indicates positively the N-fixing capacity of the fungus. Sporophores appeared from 20–25 days after inoculation of the bed and contained 34.5% protein, on dry weight basis. After the harvest of sporophores, the substrate contained protein, varying from 15 to 18%. The sporophores are tasty and delicious as a protein-rich food and the substrate with enriched nitrogen could be used as manure to crop fields. *P. sajor-caju* has also been successfully raised on the stem bits of bhendi, brinjal and papaya, mixed in equal proportions with paddy straw and wheat straw.

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## SHORT SCIENTIFIC NOTES

### Bacterial Cultures as Possible Analogues of Chemical Fertilizers

Since the pioneering work of Winogradsky<sup>1</sup>, the use of bacterial culture to ameliorate soil fertility has attracted much attention of the microbiologists. In the wake of power crisis, shortage of petroleum products, and spiralling rise in price of chemical fertilizers, researches are continuing in laboratory and field. Field measurements of  $N_2$  fixation in Californian soils have revealed dramatic rise in the activity when soils are inoculated with azotobacters or clostidia *plus* carbohydrate<sup>2</sup>.

Along with a basal amendment of phosphate, several sources of azotobacter cultures (JNKVV<sup>a</sup>, Bactogin<sup>a</sup>, Biofertilizers<sup>b</sup> and Bafe<sup>c</sup>) were applied to wheat, Narmada-4 under dryland conditions during 1973-74. The germinability and grain yield of single row treatment were noted. The Bafe brand culture could increase the germinability by 50%, and the yield by 11% in a non-significant manner (control = 738 g/5 m row). The observed increment in germinability and in yield were within the limits of field experimentation, and tallies with the report of Mishustin<sup>3</sup>.

Research data revealed that the inclusion of dimers of aldose-alonic acid mixtures is necessary for making the bacterization agronomically sound. Another trial with this type of culture prepared as described<sup>1</sup> was laid out with adequate statistical controls using combination of half and full recommended doses of nitrogenous fertilizer (15 and 30 kg N ha<sup>-1</sup>) with basal application of 30 kg P<sub>2</sub>O<sub>5</sub>/ha. Azotobacter culture was applied to the seed at the rate of 5 g/kg. Bacterization was found to be equivalent to 15 kg N ha<sup>-1</sup>, increasing the yield by 43% (control yield = 12.5 q ha<sup>-1</sup>; LSD at P = 0.05, = 3.98 q; CV = 27%), and further it was compatible with the half dose, which resulted in no additional increase. Full dose was as good as the half dose in the dry land cultivation, full dose giving 19.0 q ha<sup>-1</sup> and half dose giving 20.5 q ha<sup>-1</sup>. Because the benefit from bacterization is substantial it seemingly dispenses away the application of chemical fertilizers. Does this mean partial or complete replacement? Can bacterial cultures act as an analogue of chemical fertilizers? Compounds like gibberellins and conactins elaborated by the inoculant bacteria<sup>4</sup> are likely to insure a healthy plant stand, full of vigour drawing adequate nutrients needed for growth and production from the soil. But, whether this kind of bacterization

alters the microbial equilibrium to such an extent that nutrients are helped out from the soil or atmosphere to the agronomic benefits is largely undetermined. Incorporation of additional components into the cultures needs confirmation in a few more trials on crops. The newly formulated bacterial fertilizers on this design are currently under evaluation for their agronomic utility. Once the utility is established, the development of technology can be perfected.

Microbiology Section,

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Jawaharlal Nehru Krishi

Vishwa Vidyalaya,

Jabalpur, 482 004, December 11, 1974.

a. Jabalpur Product, b. Bombay Product, c. Poona Product.

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### Varietal Reaction to the Sheath Rot of Rice (*Acrocyndrium oryzae* Saw)

Sheath rot of rice caused by *Acrocyndrium oryzae* Sawada has been reported from Japan<sup>1</sup>, Thailand<sup>2</sup>, few countries in South East Asia<sup>3</sup> and India (Tamil Nadu and Hyderabad)<sup>4-5</sup>. The disease was noticed in a number of varieties in all seasons at the Paddy Breeding Station, Coimbatore. The present study was aimed to assess the reaction of some of the newly released and improved varieties to sheath rot under field condition.

Rice plants at booting stage in one metre square at random were examined, the number of infected and healthy plants and tillers noted and the percentage of disease incidence worked out. Dark brown to chocolate brown spots on upper sheaths resulting in either partial or incomplete emergence of the panicle and seed sterility are the chief symptoms of the disease. Almost all the grains in a sheath rot affected panicle invariably become brown in colour. The reaction of the varieties is given below:

Annapoorna (40.5); RP 4-14 (IET 2234) (33.3); Kannaki (28.5); Soorya (26.0); Cauvery (22.0); 6543 (20.0); ADT 30 (17.2); Jaya (16.8);

Manna (15-4); Kanna (15-4); 6547 (16-5); ADT 51 (15-4); Pennal (15-4); IR 26 (15-0); Kanchi (15-0); Kanchi (11-0); IR 20 (10-0); IR 5 (10-0); Sona (1ET 1991) (10-0); Bhavani (10-0); Valpar (10-0); Mala (9-6); Suhashini (9-6); Annapurna (7-2); IR 24 (7-2); IET 2222 (7-2); Tapochchi (5-0); Deegewoogen (5-0); IR (4-8); Basumathi (3-0); Chandina (2-4); Jayanthi (2-4); Kumar (2-4); Sigadis (1-0); Kanto (1-0); Tella hamsa (1-0); TKM 6 (1-0); TN 1 (1-0).

From the foregoing it can be seen that sheath rot, till now considered a disease of minor importance and unknown in Tamil Nadu, is coming into prominence. Annapoorna was comparatively more susceptible, while T.N.1, TKM 6, Tella hamsa, Kanto and Sigadis showed least susceptibility.

Tamil Nadu Agril. Univ., C. L. SUBRAMANIAN.  
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December 27, 1974.

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#### Occurrence of a New Disease on Grape Seedlings Caused by *Phytophthora nicotianae* var. *parasitica* (Dastur.) Waterhouse from India

Since 1972, severe outbreaks of a 'damping off' disease of hybrid grape seedlings have been observed at the Indian Institute of Horticultural Research, Hessaraghatta.

The disease symptoms appear as light brown, irregular water soaked areas on the collar region of the young seedlings, which soon enlarge and coalesce forming distinct depressed lesions and ultimately the seedlings collapse. Bleaching of the leaves is another initial characteristic symptom of this disease.

Microscopic examination of affected tissues from diseased seedlings repeatedly showed the presence of abundant Papillate sporangia and non-septate mycelium. The sporangia on germination produced biflagellate zoospores, characteristic of the genus *Phytophthora*. Based on the morphological and cultural characters, the fungus was identified as *Phytophthora nicotianae* var. *parasitica* (Dastur.) Waterhouse. On artificial inoculation, this fungus was found to be pathogenic on seedlings of Aster, Carnation, Bougainvillea, Petunia and Hibiscus. In addition, green fruits of tomato, okra, capsicum,

brinjal, bean, coconut, jack and guava were also found to be susceptible.

Division of Plant Pathology,  
Indian Inst. of Hort.

Research (ICAR),  
Bangalore-6, February 5, 1975.

T. S. SRIDHAR.  
B. A. ULLASAI.  
H. S. SOHI.

#### Addition to the Host Range of the New Strain of Brinjal Mosaic Virus

In the earlier report (Naqvi and Mahmood<sup>1</sup>) a new strain of brinjal mosaic virus (BMV) has been reported. On further studies, however, some Solanaceous plants were proved to be additional hosts of the virus. These are: *Solanum aculeatum* St. Lag., *S. aviculare* Forst., *S. hispidum* Pers., *S. hybridum* Jacq. and *S. mammosum* L. All the above hosts produced characteristic symptoms and the virus could be recovered on back inoculation to the test plants of *S. melongena* L. var. *Pusa Purple Long*.

Department of Botany, S. QUAMAR A. NAQVI.  
Aligarh Muslim University, K. MAHMOOD.  
Aligarh 202 001, India,  
February 28, 1975.

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#### Some Observations of Gamma Irradiation on Growth and Flowering of Tuberose Bulbs

The Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri, has initiated a programme of irradiation research on ornamental crops with a view to evolve improved strains through mutation. The present observations include data on dose-response of tuberose flowering to radiation. Some of the somatic effects are also included in this short report. Desai<sup>1</sup>, while studying effects of irradiated bulbs of tuberose with acute doses of gamma rays, reported that bulbs survived upto 2.5 Kr.

150 bulbs of single tuberose were irradiated at Bhabha Atomic Research Centre, Trombay, with 0.5 Kr., 2.5 Kr. and 3.5 Kr. doses and planted on 20-6-1974 in the horticultural Nursery of the Department of Horticulture, at Rahuri. About 20-25 bulbs of equal size were irradiated with each dose.

Data were recorded in date of appearance of first flower stalk, length of stalk at first flowering, date of first flower opening, length of flower-stalk, girth of stalk, length of internodes and the number of leaves when first flower stalk emerged.

From Table I it is seen that the gamma dose of 0.5 Kr. gave one desirable mutant with bolder

TABLE I  
Effect of gamma irradiation on growth and flowering of tuberose

| Gamma dose (Kr) | No. of days required for first flowering   | Length of stalk at first flowering (cm) | No. of days required for first opening of flower | Length of flower stalk (cm) | Girth of flower stalk (cm) | Length of internodes (cm) | No. of leaves when 1st flower stalk emerged | Other observations  |
|-----------------|--|---|--|-----------------------------|----------------------------|---------------------------|---|---|
| Control         | 150  | 40-42                                   | 156  | 70                          | 2.5-3                      | 8-10                      | 15.00                                       | ..  |
| 0.5             | 140  | 50-58                                   | 145  | 73-90                       | 1.5-4.0                    | 8-14                      | 13-30                                       | One mutant gave bolder flowers compared to control plants. Flower tube length was 5 cm compared to 4.0-4.5 cm of control and other doses. Flower diameter was 4.1 cm compared to 3.5-4 cm in control and other doses. |
| 1.5             | 140  | 42-50                                   | 148  | 69-75                       | 1.5-4.0                    | 10-14                     | 11.20                                       | 4-5 stalks were found bifurcated. Leaves with ivory coloured midrib. 3 flowers at one place instead of 2 in control and other plants.   |
| 2.5             | Very poor sprouting of bulbs. 4 out of 25 bulbs sprouted upto 5 months after planting. |   |  |                             |                            |                           |   |   |
| 3.5             | No. sprouting of bulbs.  |   |  |                             |                            |                           |   |   |

flowers compared to control plant and plants from bulbs treated with other doses. As reported by Desai the abnormalities like bifurcated stalks, ivory coloured midrib were observed in bulbs treated with 1.5 Kr gamma dose. The higher doses like 2.5 Kr and 3.5 Kr proved to be more or less lethal for sprouting tuberose bulbs.

Mahatma Phule Krishi

A. V. PATIL.

Vidyapeeth,

P. N. KALE.

Rahuri District,

S. N. KAULGUD.

Ahmednagar (M.C.),

December 6, 1975.

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### Unstable Haemoglobin

Unstable haemoglobin syndromes comprise of a group of inherited disorders characterised by haemolytic disease of varying severity. In view of paucity of reports in Indian literature, tests were conducted to detect this abnormality in 10 normal persons and 31 patients with different haematological disorders attending the Department of Haematology of the School of Tropical Medicine, Calcutta.

In control subjects, the range of heat-labile haemoglobin was 0 to 1.8%. Series of estimations

from blood samples, preserved in A.C.D. solution, revealed a gradual rise of unstable haemoglobin level reaching 4% on the 10th day and thereafter a fall reaching normal level after two weeks.

Two cases of unstable-haemoglobin syndromes were detected; One had 32% of unstable haemoglobin with mild haemolytic anaemia and the other with 12.5% of unstable haemoglobin associated with iron deficiency anaemia.

Excess amounts of heat-labile haemoglobin upto 12% range were detected in 10 out of 16 cases with Haemoglobin-E-Thalassaemia disease. It is probably from the excess alpha chains synthesised by the reticulocytes and young red cells.

Heat-labile haemoglobin was not detected in other haematologic disorders such as, iron deficiency anaemia, glucose-6-phosphate dehydrogenase deficiency, idiopathic thrombocytopenic purpura and myelophthisic anaemias.

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Cuttack 753 007, November 21, 1974.

### Occurrence of *Pythium butleri* Subr. in *Amaranthus tricolor* var. *gangeticus* (Linn.) F and P.

A severe 'damping off' disease of amaranthus [*Amaranthus tricolor* var. *gangeticus* (Linn.) F & P.] seedlings was observed in November 1973, around



Hebbal, Bangalore, the disease was noticed when the seedlings were in 4-6 leaf stage. Affected plants showed rotting of the stem at ground level. In the initial stages, water-soaked zone at basal portion was noticed which later turned brown and soft. The infected portion got constricted resulting in toppling of the seedlings. The seedlings remained in this condition for one or two days and later died. The disease was observed to occur in patches. Repeated isolations from the diseased seedlings in culture yielded a pythiaceus fungus.

For pathogenicity studies, the fungus, cultured on corn-meal agar, was mixed with sterilized soil in plastic trays. A week later, surface sterilized amaranthus seeds were sown into them. Both pre and post-emergence 'damping off' was noticed at germination time in inoculated soil. Out of 300 seeds sown 268 seeds germinated in uninoculated soil as against 64 in inoculated soil. All the seedlings in inoculated soil died in a fortnight's time. The pathogen was reisolated from such infected seedlings. The fungus was identified at Centraal-

bureau Voor Schimmelcultures, Baarn, Netherlands, as *Pythium butleri* Subramanian. The culture has been deposited in the culture collection of Department of Plant Pathology, U.A.S., Bangalore, and has been given accession No. 101.

The fungus is known to attack tobacco, ginger, papaya, chille<sup>1</sup>, maize<sup>2</sup>, and torai<sup>3</sup>. However, there is no record of this fungus on amaranthus and this is a new host record.

Grateful thanks are due to Director and Dr. R. A. Samson of CBS, Netherlands, for identification of the fungus, Dr. H. C. Govindu, Sr. Professor and Head of the Department, for facilities, Dr. N. P. Patil, Director of Research, for encouragement.

Dept. of Plant Pathology, H. R. REDDY.  
College of Agri., U.A.S., T. B. ANILKUMAR.  
Hebbal, Bangalore 560 024.  
November 26, 1974.

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## REVIEWS AND NOTICES OF BOOKS

### Annual Review of Physical Chemistry (Vol. 25).

Edited by H. Eyring, C. J. Christensen and H. S. Johnston. (Annual Reviews, Inc., 4139 Camino Way, Palo Alto, California 94306). Pp. 566. Price : U.S.A., \$ 12.00 ; Elsewhere \$ 12.50.

The book contains 19 chapters on a variety of topics in physical chemistry. Starting from the history of physical chemistry in Denmark which forms the first chapter, the book covers several subjects of current interest and development, e.g., laser light scattering from liquids, laser-induced chemical reactions, lipid phases, mechanism of ion-transport in lipid membranes, magnetic circular dichroism to mention a few.

In the chapter on the history of chemistry in Denmark, the author Prof. T. A. Bak describes some important discoveries made in Denmark but it appears as though nothing much has been achieved after 1947.

Studies on lipid-water systems with particular emphasis on X-ray scattering have been described thoroughly by Luzzati and Tardieu. Structures of the phases are illustrated with the help of impressive diagrams.

The instrumentation, the general theory and the applications of the method of magnetic circular

dichroism are discussed and the literature coverage is from 1960 to 1973.

Laser light scattering studies as applied to macromolecules bacterial motion, gels, collision induced anisotropies and rotational motion of molecules in liquids have been described. An account of infrared- and ultraviolet-laser induced reactions, selective two step reactions and laser temperature jump relaxation experiments is given. The areas of vibrational and rotational relaxation in the ground state of small molecules using the laser devices and of the molecular trajectory calculations have been covered. Recent developments relating to the understanding of molecular collision have been given. The basis of the electrical excitability of nerve cells has been discussed in the chapter on excitable membranes. Time domain electron paramagnetic resonance, electron-nuclear double resonance of free radicals in solution and of proteins and electron-electron double resonance in organic systems have been exhaustively reviewed. Experimental techniques, their objectives and the scope for studies on gaseous negative ions, theory of liquid mixtures of non-electrolytes and the spectroscopy of linear polyenes have been described. Vibration relaxation in condensed media and its

role in unimolecular reactions and molecular luminescence, the present state of the subject of oscillatory chemical reactions, the theory of  $\pi$ -molecular charge transfer crystals and their magnetic, optical and electric features are covered. Developments in the field of rotation and rotation vibration pressure broadened spectral line shapes have been described with particular emphasis on the work after 1967. A critical analysis of the controversies in the literature on polymer statistical mechanics has been made.

At the end cumulative indices of the contributing authors and the chapter titles of volumes 21 to 25 of the series are given.

The overall coverage of the literature is quite up-to-date and the section of the topics is, in general, of considerable interest to physical chemists.

C. L. KHETRAPAL.

#### Quantum Mechanics in Chemistry (2nd Edn.).

By Melvin W. Hanna. (W. A. Benjamin Inc., Advanced Book Programme, Reading, Mass.), 1973. Pp. xv + 260. Price not given.

This book is an attempt to introduce to the students of chemistry certain aspects of quantum mechanics that should be known to every chemist. However, none of these aspects is discussed in full and the author has very rightly confessed in his preface that students using this book need to spend considerable time in the library reading other works. Although most chapters are written with greater clarity than those in the previous edition there is much to be done yet within the limits of this book.

In the chapter on spectroscopy, the author has discussed the interaction of radiation with atom or molecule but refrained from discussing the time-dependent perturbation theory by saying that the latter is mathematically more complicated than the time-independent perturbation theory. But, I should imagine that a simplified quantitative treatment of this topic should not have been beyond the scope of this book. This aspect of quantum mechanics is very important because it forms the basic foundation of spectroscopy.

In the chapter on molecules and the chemical bond, the symbols  $\sigma$ ,  $\pi$ ,  $\delta$ , etc., and  $\Sigma$ ,  $\Pi$ ,  $\Delta$ , etc., are not explained while they are freely used in several pages.

Chapter 9 is readable and interesting and provides enough material to build a foundation in the chemical aspect of the magnetic resonance spectroscopy.

This book can be recommended as a companion volume in physical chemistry for the students of chemistry at the University level in India.

A. K. CHANDRA.

#### Books Received

*Advances in Plant Morphology.* Edited by Y. S. Murty, B. M. Johri, H. Y. Mohan Ram and T. M. Varghese. (Sarita Prakashan, 175, Nauchandi Gardens, Meerut, U.P.), 1974. Pp. xvi + 447. Price Rs. 100.00.

*Biology of the Land Plants.* Edited by V. Puri, Y. S. Murty, P. K. Gupta and D. Banerji. (Sarita Prakashan, 175, Nauchandi Gardens, Meerut, U.P.), 1974. Pp. xi + 433. Price Rs. 120.00.

*Particles Sources and Fields.* By Julian Schwinger. (Addison-Wesley/W. A. Benjamin, Inc., Advanced Book Programme, Reading, Massachusetts 01867), 1973. Pp. 459. Price \$ 18.50.

*System Identification Methods and Applications—Methods and Applications.* By Harriet H. Hagiwada. (Addison-Wesley/W. A. Benjamin, Inc., Advanced Book Programme, Reading, Massachusetts 01867), Pp. xix + 293. Price \$ 16.00 (Cloth); \$ 8.50 paper.

*Fortschritte Der—Experimentellen Und Theoretischen Biophysik.* (Band 17) (VEB Georg Thieme, Absatzabteilung, DDR 69, Jena, Villengang-2), Pp. 117. Price 30 M.

*Modern Microscopy—Elementary Theory and Practice.* By C. F. A. Culling. (Butterworths & Co., Ltd., 88, Kingsway, London WC 2 B 6 AB), 1974. Pp. xii + 148. Price £ 1.50.

*Treatment of Inborn Errors of Metabolism, Current Treatment and Future Prospects.* Edited by J. W. T. Seakins, R. A. Saunders and C. Toothill. (Churchill Livingstone, Edinburgh), 1973. Pp. xiv + 260. Price £ 7.00.

*Thermal Analysis.* By Aronin Blazek. (Van Nostrand Reinhold Co., Ltd., 25-28, Buckingham Gate, London S.W. 1), 1972. Pp. 286.

*MTP International Review of Science—Defence and Recognition.* (Biochemistry Series One, Vol. 10). Edited by R. R. Porter. (Butterworths, 88, Kingsway, London WC 2 B, 6 AB), 1973. Pp. 419. Price \$ 19.50; £ 8.50 net in UK only.

#### A CORRECTION

*Current Science.* 1974, 43, page 700, Note on *Rain Tree Fruit*: for *Albezzia lebbeck* (Dirisana in Telugu) read *Samania saman* (Jacq.) Meer. (Nidraganneru in Telugu).

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# FISSION TRACK AGES AND URANIUM CONCENTRATION OF APATITES OF DIFFERENT ROCKS OF SOUTH INDIA

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## ABSTRACT

The uranium concentration and ages of apatite grains of various rocks of South India have been measured by fission track technique. The ages range from 100 m.y. to 730 m.y. whereas uranium concentrations vary from 0.5 to 23.8 atom/million atoms of the apatite mineral. The ages agree well with the Deccan volcanic and Indian Ocean Cycle activities.

## INTRODUCTION

THE fission track technique of dating the minerals is a promising tool for geochronological studies. Since the discovery of track etch technique by Fleischer and Price<sup>1</sup>, Nagpaul *et al.*<sup>2</sup>, and Nand Lal *et al.*<sup>3</sup> dated some of the Himalayan granites, Bundelkhand granites<sup>4</sup> and alkaline rocks of India<sup>5</sup> by 'in situ' apatite measurements. The present report on South India is in continuation of the same. The results have been discussed in the light of other isotopic age data available for the region.

## EXPERIMENTAL PROCEDURE AND RESULTS

Transparent sections of about 1 to 2 cm<sup>2</sup> area and 100–200 micron thick rock specimen were prepared by polishing successively with 800, 1200,

1500 mesh of aluminium oxide abrasive and finally with 8 micron diamond paste. They were etched with 5% HNO<sub>3</sub> at about 25° C for 30–50 seconds to reveal the fission tracks in apatite grains. Measurements of fossil and induced densities were carried out by usual procedures described elsewhere<sup>2</sup>. No observations were made (i) when grain size was very small (< 50 microns), (ii) there was non-uniformity of track density in the grain, (iii) near the grain boundary. The thermal neutron flux  $\phi$  was measured by counting  $\sim 10^4$  tracks in the uranium loaded glass sample irradiated simultaneously with the rock sections. The age T and uranium concentration  $C_w$  calculated with the following relations are given in columns 6 and 9 respectively in Table I.

TABLE I

*Fission track ages and uranium concentration in apatites*

| S. No. | Name and Location of Rock  | No. of analysis made | Fossil track density ( $\rho_g$ ) $\times 10^6/\text{cm}^2$ | $\rho_g/\rho_i$ | Observed fission track age (million years) | Track length reduction % (corresponding density reduction) | Corrected f.t. age (million years) | U-conc. atom/ $10^6$ atom | Total neutron dose $\times 10^{16}$ |
|--------|--|----------------------|---|-----------------|--|--|------------------------------------|---------------------------|-------------------------------------|
| 1      | 2  | 3                    | 4   | 5               | 6  | 7  | 8                                  | 9                         | 10                                  |
| 1.     | Granite from Tharumarakuppan hill near Puttur (A.P.)                   | 3                    | 2.6   | .473            | 600 $\pm$ 60                               | a  | 600 $\pm$ 60*                      | (0.5–1.7)                 | 2.2                                 |
| 2.     | Granite from Khamam Dist. (A.P.)                                       | 4                    | 5.5   | .293            | 580 $\pm$ 60                               | a  | 580 $\pm$ 60*                      | (1.5–3.5)                 | 3.5                                 |
| 3.     | Biotite granite gneisses from Kottavalasa, Vishakhapatnam Dist. (A.P.) | 4                    | 8.2   | .257            | 470 $\pm$ 5                                | a  | 470 $\pm$ 5*                       | (3.6–6.0)                 | 3.0                                 |

TABLE I—Contd.

| 1   | 2   | 3 | 4   | 5    | 6       | 7        | 8       | 9          | 10  |
|-----|---|---|-----|------|---------|----------|---------|------------|-----|
| 4.  | Hornblende grano-<br>diorite from<br>Gudur-Nellore<br>schist belt.  | 3 | 3.4 | .660 | 460±60  | 7 (3)    | 480±60  | (1.7)      | 1.2 |
| 5.  | Hornblende pyro-<br>xene granulite<br>from Kottavalasa<br>Vishakhapatnam<br>Dist. (A.P.)  | 8 | 6.5 | .480 | 370±40  | 32 (32)  | 490±70  | (2.2-12.5) | 1.3 |
| 6.  | Coarse biotite<br>hypersthene<br>gneisses from<br>Pre-cambrian<br>terrain of Kotta-<br>valasa, Vishakha-<br>patnam Dist. (A.P.) | 6 | 6.8 | .691 | 480±70  | 9 (3)    | 500±75  | (2.2-3.5)  | 1.2 |
| 7.  | Granite from<br>Krishan Samud-<br>ram Tunvola near<br>Chittoor (A.P.)   | 3 | 2.2 | .271 | 340±30  | 20 (16)  | 410±40  | (1.0-2.0)  | 2.2 |
| 8.  | Nirth Granite<br>(A.P.)   | 3 | 3.5 | .230 | 300±20  | 21 (18)  | 360±30  | (1.4-4.0)  | 2.2 |
| 9.  | Nepheline Syenite<br>from Kundulura<br>(17° 40'; 81° 24')<br>(A.P.)   | 5 | 2.9 | .245 | 250±30  | 36 (38)  | 400±50  | (0.8-4.4)  | 1.7 |
| 10. | Hornblende Syenite<br>from Phanai<br>Mata Hill,<br>Chhota Udaipur<br>(Gujarat)  | 4 | .4  | .095 | 100±10  | <i>a</i> | 100±10* | (0.8-1.4)  | 1.7 |
| 11. | Granite from<br>Attur, South<br>Arcot Dist. (T.N.)  | 3 | 4.5 | .378 | 670±30  | <i>a</i> | 670±30* | (4.4-23.8) | 3.0 |
| 12. | Biotite granite,<br>5-6 km from<br>Arsikere<br>(Karnataka)  | 3 | 20  | .332 | 730±80  | <i>a</i> | 730±80* | (4.2-9.4)  | 3.8 |
| 13. | Charnockite rock<br>from Kabbal<br>South of<br>Bangalore<br>(Karnataka)   | 5 | 7.5 | .749 | 730±115 | 11 (4)   | 760±120 | (1.7-3.9)  | 1.7 |

*a* Annealing correction to these ages was not possible.

\* Uncorrected ages.

$$T = 6.57 \times 10^9 \ln (1 - 9.25 \times 10^{-15} \frac{\rho_s}{\rho_i} \phi, \text{ yrs.})$$

$$C_x = 4.0 \times 10^8 \rho_i \phi \text{ atom/atom}$$

where  $\rho_s$  = fossil track density

$\rho_i$  = induced track density

$\phi$  = integrated thermal neutron dose.

In order to assess the geological annealing, *in situ* length measurements of fossil tracks were made<sup>6</sup> and compared with the length of induced fission tracks. The method for applying the corrections

has already been discussed in the literature<sup>7</sup>. The corrected ages are given in column 8 of Table I. The corrections to some of the ages could not be made because (i) fossil track density  $< 10^6/\text{cm}^2$ , does not satisfy TINT formation conditions<sup>6</sup> as in sample No. 10. (ii) Number or size of apatite grains, though enough for age determinations, was not sufficient to give statistically significant number of TINTS. The errors given with the individual values are only the statistical counting errors com-

puted from fossil and induced track densities. The mean values are given with one standard deviation. Measurements of fossil and induced track densities on the same grain, no doubt eliminates the error due to the non-uniformity of uranium on the surface, but the variation of the uranium in the volume may give rise to some errors in the age. This factor along with the uncertainty in the neutron dose measurement constant, have not been quoted with the ages. The uranium content in apatite shows large dispersion. Even in the same section it varies from grain to grain.

### DISCUSSION

In general, the ages of Andhra Pradesh witnessed the influence of Eastern Ghat belt which runs from Cuttack to Bezwada, attaining the maximum width in Cuttack-Ganjam region<sup>8</sup>. The dominant trend of the belt in Andhra Pradesh is NE-SW, with local variation due to cross folding<sup>9-11</sup>. It is mainly composed of sub-parallel alternating layer of khondalites and their variants, granite gneisses and charnockites<sup>8</sup> (pyroxene granulite). On the basis of available geological and geochronological data, the following probable sequence of events can be assigned to this belt<sup>8,12,13,14</sup>.

(i) Deposition of pelitic sediments in an extensive geosyncline.

(ii) Folding and metamorphism of the sedimentary rocks to form khondalites (~1600 m.y.).

(iii) Emplacement of charnockites and granites (~1300-1500 m.y.).

(iv) Metamorphism, folding and uplift of the Eastern Ghats (~700-450 m.y.) known as Indian Ocean cycle.

The fission track ages of different apatites (Andhra Pradesh) range from 360 to 600 m.y. (sample Nos. 1 to 9). These ages which generally respond to last metamorphic activity due to high sensitivity of fission tracks against thermal variations support the last, i.e., Indian Ocean cycle episode (~700-450 m.y.)<sup>13</sup>. The mineral under investigation is apatite which is most temperature sensitive (as far as fission tracks are concerned), further strengthen the susceptibility of these fission track ages to the last thermal event. These fission track ages of apatites of Andhra Pradesh, also fall in the range of fission track ages of minerals muscovite, biotite and apatite of Nellore mica belt<sup>15</sup>.

The age ( $580 \pm 60$  m.y.) of Khamam Dist. is comparable with the isotope age of Galena (650-770 m.y.)<sup>12</sup>. It is also consistent with the other fission track ages made on zircon ( $518 \pm 17$  m.y.) and apatite ( $571 \pm 37$  m.y.) minerals of pegmatitic origin<sup>16</sup> from the nearby regions.

The age  $100 \pm 10$  m.y. of hornblende synetite of Phanai Mata Hill indicates that it lies within Deccan Volcanic province. On the basis of this result, it should be placed well within Mesozoic.

The fission track age  $670 \pm 30$  m.y. of granite from Tamil Nadu (sample No. 11) is corroboratable with 700 m.y. event, i.e., Indian Ocean cycle.

Karnataka region has undergone quite a number of orogenic metamorphic cycles, the effect of which is found to be increasing towards the south, accompanied by some igneous activity (pegmatization, granitization, and alkaline gabbro syenite complex)<sup>8</sup>. The fission track ages  $730 \pm 80$  and  $760 \pm 120$  m.y. of sample Nos. 12 and 13 are probably due to latest thermal event during Indian Ocean cycle or due to some later igneous activity. Due to the low annealing temperature, the age  $730 \pm 80$  of apatite is lower than the corresponding fission track age of hornblende ( $840 \pm 33$  m.y.) from the same region<sup>17</sup>.

### CONCLUSION

From the above data it may be concluded that : most of the fission track ages of apatite grains from different rocks of South India corroborate the Deccan Volcanic and Indian Ocean cycle.

### ACKNOWLEDGEMENT

We are thankful to Council of Scientific and Industrial Research, New Delhi and PL-480 NBS (G) 182 research grant for financial assistance.

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## OXOTITANIUM(IV) COMPLEXES WITH 2-OH-ACETOPHENONE OXIMES

N. S. BIRADAR,\* M. D. PATIL AND V. B. MAHALE

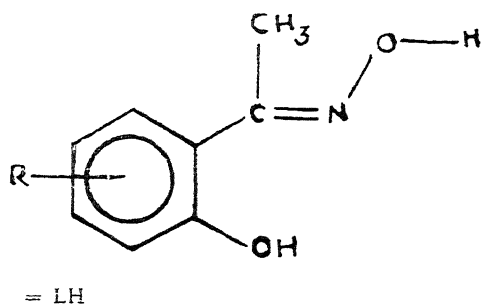
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## ABSTRACT

Oxotitanium (IV) complexes have been prepared by treating oxotitanium (IV) perchlorate with 2-OH-acetophenone oxime in aqueous alcohol medium. The complexes are orange-yellow to orange-red in colour. These complexes are characterised by elemental analysis, molecular weight, conductometric and spectral data. Five coordinate trigonal bipyramidal structure has been suggested for these complexes.

## INTRODUCTION

COMPLEXES with oximes have been the subject-matter of a large number of investigators<sup>1,2</sup>. This paper deals with a study of complexes of oxotitanium (IV) with the following ketoximes (I-V).



## EXPERIMENTAL

2-OH-acetophenone and substituted 2-OH-acetophenones were prepared by known method<sup>3</sup>. Hydroxylamine hydrochloride was of reagent grade. The ketoximes were prepared by heating a solution of 1 g of 2-OH-acetophenone, 1 g of hydroxylamine hydrochloride and 2 g of sodium acetate in 10 ml of ethanol on a steam bath for an hour. The ketoximes thus prepared were recrystallised from aqueous ethanol. Titanyl perchlorate was prepared according to the method reported in the literature<sup>4</sup>.

Complexes were prepared by reacting oxotitanium (IV) perchlorate (10 m mole) dissolved in aqueous ethanol with ketoxime (22 m mole) in aqueous ethanol with vigorous shaking. Sufficient time was allowed for the precipitate to settle. The complex formed was filtered, washed with aqueous ethanol and dried at 110° C.

The complexes were analysed for titanium and nitrogen contents by standard methods. The molecular weights of the complexes in nitrobenzene were determined by the Beckman freezing point method. The infrared spectra of the complexes and the ligands in nujol mull were recorded on Perkin-Elmer-337 in the region 4000-400 cm<sup>-1</sup>.

## RESULTS AND DISCUSSION

The elemental analysis of the complexes (Table I) show that oxotitanium (IV) perchlorate reacts with ketoxime in 1:2 ratio, losing two of its perchlorate ions. The complexes are orange-yellow to orange-red in colour and highly soluble in common organic solvents. Molecular weights of the complexes determined in nitrobenzene agree well with the empirical formulae and the monomeric nature in nitrobenzene. These complexes show negative test for perchlorate ion. The molar conductance values in nitrobenzene at 10<sup>-3</sup> M fall in the range 0-3.0 ohm<sup>-1</sup> cm<sup>2</sup>/mole, indicating that these are nonelectrolytes in nitrobenzene.

**Infrared Spectra.**—The important infrared frequencies are given in Table II along with their assignments. The IR spectra of the ligands under investigation show two bands, one around 3340 cm<sup>-1</sup> and other around 2600 cm<sup>-1</sup>; the former is broad with high intensity and the latter is weak. The band around 3340 cm<sup>-1</sup> is due to intermolecular hydrogen bonded -OH, and that around 2600 cm<sup>-1</sup> to intramolecular hydrogen bonded -OH. In case of salicylaldoximes the band due to intramolecular hydrogen bonded -OH is observed around 3200 cm<sup>-1</sup>. This shows that the intramolecular hydrogen bonding in ketoximes is stronger than that present in salicylaldoximes. In the complexes the band at 2600 cm<sup>-1</sup> vanishes and the band around 3340 cm<sup>-1</sup> is retained, which indicates the presence of intermolecular hydrogen bonding -OH.

In view of the previous assignments<sup>5,6</sup> the high intensity band in the region 1640-1630 cm<sup>-1</sup> in ligands is attributed to C=N stretch. In complexes this band is observed in the region 1550-1530 cm<sup>-1</sup>. This shift towards the lower frequency indicates the coordination of the C=N group to oxotitanium,

\* For correspondence.

TABLE I  
Elemental analysis of oxotitanium (IV) complexes with 2-OH-acetophenone oximes

| No. | Complex   | Empirical formulae  | Ti%   |        | N%    |        | Molecular weight<br>by cryoscopic<br>method |        |
|-----|---|---|-------|--------|-------|--------|---|--------|
|     |   |   | Found | Calcd. | Found | Calcd. |   |        |
|     |   |   |       |        |       |        | Found                                       | Calcd. |
| 1.  | Bis (2-OH-acetophenone oximate) oxotitanium (IV)      | TiO (C <sub>8</sub> H <sub>8</sub> O <sub>2</sub> N) <sub>2</sub>   | 12.90 | 13.18  | 7.63  | 6.69   | 375   | 364    |
| 2.  | Bis (3-Me-2-OH-acetophenone oximate) oxotitanium (IV) | TiO (C <sub>9</sub> H <sub>10</sub> O <sub>2</sub> N) <sub>2</sub>  | 12.03 | 12.25  | 7.20  | 7.14   | 412   | 392    |
| 3.  | Bis (4-Me-2-OH-acetophenone oximate) oxotitanium (IV) | TiO (C <sub>9</sub> H <sub>10</sub> O <sub>2</sub> N) <sub>2</sub>  | 12.40 | 12.25  | 7.05  | 7.14   | 410   | 392    |
| 4.  | Bis (5-Me-2-OH-acetophenone oximate) oxotitanium (IV) | TiO (C <sub>9</sub> H <sub>10</sub> O <sub>2</sub> N) <sub>2</sub>  | 11.95 | 12.25  | 7.26  | 7.14   | 415   | 392    |
| 5.  | Bis (5-Cl-2-OH-acetophenone oximate) oxotitanium (IV) | TiO (C <sub>8</sub> H <sub>7</sub> O <sub>2</sub> NCl) <sub>2</sub> | 12.10 | 11.09  | 6.56  | 6.46   | 446   | 433    |

TABLE II  
Infrared spectra (cm<sup>-1</sup>) of complexes and ligands

| Sl. No. | Intermolecular<br>hydrogen bonded<br>-OH |             | Intramolecular<br>hydrogen bonded<br>-OH |         | $\gamma$ C = N |         | Phenolic C-O |         | $\gamma$ Ti=O | $\gamma$ M-N | $\gamma$ M-O |
|---------|--|-------------|--|---------|----------------|---------|--------------|---------|---------------|--------------|--------------|
|         | Ligand                                   | Chelate     | Ligand                                   | Chelate | Ligand         | Chelate | Ligand       | Chelate | Chelate       | Chelate      | Chelate      |
| I       | 3340<br>b.s                              | 3300<br>b.w | 2600<br>b.w                              | ..      | 1635 s         | 1535 s  | 1290 s       | 1310 s  | 1020<br>b.m   | 520 s        | 425 s        |
| II      | 3330<br>b.s                              | 3300<br>b.w | 2650<br>b.w                              | ..      | 1630 s         | 1535 s  | 1285 s       | 1300 s  | 1025<br>b.m   | 523 s        | 455 s        |
| III     | 3340<br>b.s                              | 3300<br>b.w | 2650<br>b.w                              | ..      | 1635 s         | 1550 s  | 1290 s       | 1310 s  | 1020<br>b.m   | 520 s        | 430 s        |
| IV      | 3345<br>b.s                              | 3300<br>b.w | 2650<br>b.w                              | ..      | 1638 s         | 1538 s  | 1285 s       | 1300 s  | 1040<br>b.m   | 530 s        | 420 s        |
| V       | 3330<br>b.s                              | 3300<br>b.w | 2650<br>b.w                              | ..      | 1635 s         | 1538 s  | 1295 s       | 1310 s  | 1030<br>b.m   | 580 s        | 480 s        |

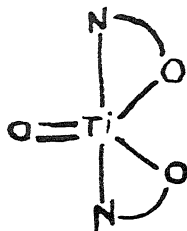
through nitrogen and lowering of the bond order of the carbon to nitrogen link. The strong band in the region 1280–1300 cm<sup>-1</sup> of the ligands, due to the phenolic C–O is found in the region 1300–1310 cm<sup>-1</sup> in the complexes. This is indicative of titanium-oxygen bond formation with oxygen of the *o*-OH group of the ketoximes.

It is evident from the previous reports<sup>7,8</sup>, that a discrete Ti=O group in the complexes gives a sharp band in the multiple bonded metal-oxygen stretching region, 1100–900 cm<sup>-1</sup>. In all the complexes studied, a high intensity broad band is observed in the region 1040–1020 cm<sup>-1</sup>. This is attributed to the Ti=O stretching vibration,

The metal-nitrogen bands are reported to occur in the range 600–500 cm<sup>-1</sup> for the Schiff base<sup>9</sup> and oxime<sup>1</sup> complexes. In view of these assignments, we have assigned the band in the region 560–520 cm<sup>-1</sup> to (Ti–N) vibration. The presence of the only one band in these complexes suggests that the complexes exist in the *trans* form. The region 500–400 cm<sup>-1</sup> is attributed to M–O stretching vibrations<sup>10,11</sup> in the complexes. In the light of these observations a medium intensity band found around 400 cm<sup>-1</sup> is assigned to (M–O) vibration.

All these observations suggest that these oxotitanium (IV) complexes have coordination number five. On the basis of the previous literature<sup>12</sup> a

trigonal bi-pyramidal structure with intermolecular hydrogen bonding is proposed for the present series.



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### PREVALENCE OF SOLUBLE COMPLEMENT FIXING (SCF) ANTIBODY TO JAPANESE ENCEPHALITIS (JE) VIRUS IN CASES OF JE VIRUS INFECTION

JAGDISH RAI,\* N. P. GUPTA\*\* AND S. N. GHOSH†

#### ABSTRACT

Serial samples of human sera belonging to confirmed cases of Japanese encephalitis (JE) virus infection were studied for the presence of antibody to soluble complement fixing (SCF) antigen of JE virus.

All the convalescent phase sera (39) out of a total of 44 sera tested, were positive for JE SCF antibodies whereas all the acute phase sera (5) were negative for these antibodies. There was complete correlation between results of complement fixation (CF) and Agarose gel diffusion (AGD) tests with JE SCF antigens. Use of JE SCF antigen as a useful reagent for specific serodiagnosis of JE virus infection by CF and AGD tests is suggested.

FALKLER *et al.* (1973) detected SCF antibodies exclusively in sera of secondary dengue cases in convalescent phase of the infection. They were unable to detect SCF antibodies in acute and primary convalescent phase sera of patients with dengue infection.

Presence of SCF antibodies in human sera in Japanese encephalitis (JE) virus infection has been demonstrated recently (Rai *et al.*, 1975) by complement fixation (CF) and Agarose gel double diffusion (AGD) tests. However, only a limited

number, mostly secondary type convalescent phase sera were found to be positive for JE SCF antibodies.

In view of the absence of correlation between HI, CF and/or neutralizing antibodies and SCF antibodies, more sera from confirmed cases of JE virus infection were tested.

#### MATERIAL AND METHODS

*Viruses*.—JE Virus (VRC Strain P 20778) plaque purified in Vero cells was used for preparation of JE SCF antigen. SCF antigens of West Nile (Strain G 22886), DEN-1 (Hawaii), DEN-2 (Strain P 23085 and TR 1751), DEN-3 (Strain 633798), DEN-4 (Strain 642069 and 611319) and Chikungunya (CHIK strain 634029) viruses were also prepared.

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**SCF Antigens.**—SCF antigens were prepared from the infected mouse brain. A 20% suspension of infected brains of the suckling mice in 0.02 M Tris HCl buffer (pH 8.2) was centrifuged at  $105,000 \times G$  for 4 hours and upper third of the supernatant fluid was used as SCF antigen. The control SCF antigen was prepared in a similar manner from the brains of normal infant mice inoculated with plain normal saline (0.02 ml each, intracerebrally).

**Immune Peritoneal Fluids.**—Mouse hyperimmune peritoneal fluids to crude mouse brain suspensions of JE (P 20778), WN (strain G 22886), DEN-1 (Hawaii), DEN-3 (Strain 633798) and Chikungunya (CHIK) viruses and to SCF antigens of these viruses were prepared in adult female mice according to the method described by Brandt *et al.* (1967).

**Human Sera.**—The sera used in the present study were collected during an outbreak of Japanese encephalitis (JE) virus infection in North Arcot District of Tamil Nadu State, India in 1955 and 1956 and stored at Virus Research Centre, Poona. A total of 44 sera belonging to 14 cases of JE virus infection confirmed clinically and serologically (by HI, CF and/or neutralization tests) as reported earlier (Webb and Pereira, 1956 and Work and Shah, 1956) were available. The sera had been kept all these years at  $-20^{\circ}C$ . These 44 sera were comprised of serial samples, collected during acute and convalescent phases of illness. Sera collected within 7 days after the onset of symptoms were referred to as acute samples whereas those collected after 8 days were considered as convalescent sera.

**Complement Fixation Test.**—CF tests were done by employing the procedure described by Casals (1967) adapted to micro-techniques in 'U' plates employing 4–8 units of SCF antigen per test volume (0.025 ml).

**Agarose Gel Diffusion (AGD) tests.**—Double diffusion immunoprecipitation tests were performed on  $7 \times 2.5$  cm microscopic slides employing 1% agarose in 0.05 M borate saline (pH 9.0). Merthiolate (1:10,000) or sodium azide (1:1000) was used as preservative. About 3.0 ml melted agarose (1%) was sufficient for each slide. Wells were cut in hexagonal pattern (one central well having six peripheral wells) with 4 mm diameter and 8 mm centre to centre distance. SCF antigens, standardized to 256 units, and normal antigen were placed in central wells and the human sera in the peripheral wells.

## RESULTS

Clinico-serological classification of the sera tested and results of CF and AGD tests with JE SCF antigen are given in Table I. These results show that all the convalescent phase sera (39) were positive for JE SCF antibodies in CF and AGD tests. The earliest time of detection of JE SCF antibodies was 9 days after the onset of symptoms. Out of these 39 sera positive for JE SCF antibodies 7 sera were of monoreacting type (antibodies against JE virus only) and 32 sera were of cross reacting (secondary) type, showing cross reactions with sucrose acetone extracted mouse brain (SAMB) antigens of other group B arboviruses also. All the 5 acute phase sera were negative for JE SCF antibodies in CF and AGD tests. There was no correlation between antibodies to SAMB antigens and SCF antibodies. Some of the sera which had high HI and CF antibodies to SAMB antigens had low JE SCF antibody titres and *vice versa* too. In one case of late convalescent phase (352 days after onset of symptoms) there were no detectable HI and CF antibodies to SAMB antigens whereas JE SCF antibodies were detected in CF and AGD tests. These 39 sera positive for JE SCF antibodies did not react with SCF antigens of West Nile (WN), dengue (DEN 1-4) and Chikungunya (CHIK) viruses and normal antigen in CF and AGD tests.

## DISCUSSION

JE SCF antibodies were detected in all the convalescent phase sera (39), whether nonreacting (1) monoreacting (7) or cross reacting (31) types. On the other hand all the acute phase sera (5), whether nonreacting (2) or monoreacting (2) or cross reacting (1) types were negative for JE SCF antibodies. There was complete correlation between results of CF and AGD tests with JE SCF antigen. All the 39 sera showing JE SCF antibodies in CF tests were also positive in AGD tests with JE SCF antigen, but none of them reacted with SCF antigens of WN, DEN (1-4) and CHIK viruses and normal antigen in CF and AGD tests. These findings have demonstrated a considerable degree of specificity and sensitivity of JE SCF antigen and antibody in CF and AGD tests.

It is too premature to comment on the role of SCF antigen and the significance of SCF antibody in JE virus infection. However, the specificity and sensitivity of JE SCF antigen seen in the serological reactions (CF and AGD tests) suggests that it could be employed as a useful reagent for specific serodiagnosis of JE virus infection.

TABLE I

*Antibodies to JE SCF antigen in human sera during an outbreak of JE virus infection in Tamil Nadu (then Madras State) India (1955-1956)*

| *Type of sera<br>(Clinico-serological<br>classification) | No. of<br>sera<br>tested | *No.<br>positive for<br>antibodies<br>to JE and<br>other group<br>B arbo-<br>viruses<br>(cross-<br>reacting) | *No.<br>positive for<br>antibodies<br>to JE virus<br>only<br>(Mono-<br>reacting) | *No.<br>non-reacting<br>to any of<br>the group B<br>arboviruses<br>antigens<br>(Non-<br>reacting) | No.<br>positive for<br>JE SCF<br>antibodies<br>in CF tests | †No.<br>positive for<br>AGD-test<br>with JE<br>SCF<br>antigen |
|--|--------------------------|--|--|---|--|---|
| 1. Acute non reacting ..                                 | 2                        | 0  | 0  | 2   | 0  | 0   |
| 2. Acute monoreacting ..                                 | 2                        | 0  | 2  | 0   | 0  | 0   |
| 3. Acute crossreacting ..                                | 1                        | 1  | 0  | 0   | 0  | 0   |
| 4. Convalescent nonreacting ..                           | 1                        | 0  | 0  | 1   | 1  | 1   |
| 5. Convalescent monoreacting ..                          | 7                        | 0  | 7  | 0   | 7  | 7   |
| 6. Convalescent crossreacting ..                         | 31                       | 31   | 0  | 3   | 31   | 31  |
| TOTAL ..   | †44                      | 32   | 9  | 3   | 39   | 39  |

\* As determined by SAMB antigens of JW, WN, DEN 1-4 and MVE viruses in HI, CF and/or N tests. Serum samples collected upto 7th day after the onset of symptoms were classified as acute phase sera and those collected after 7 days were considered as convalescent phase sera.

† These 44 sera comprised of serial samples collected during acute and convalescent phases from 14 confirmed cases of JE virus infection.

‡ Sera positive for JE SCF antibodies gave precipitin lines in AGD tests with JE SCF antigen only and not with WN SCF antigen or normal antigen.

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## LETTERS TO THE EDITOR

**EFFECT OF ADDED COMPLEXING AGENTS AND BASES ON THE RATE OF OXIDATION OF  $\alpha$ -HYDROXY ACIDS BY LEAD TETRA ACETATE**

The rate of cleavage of 1, 2 diols by lead tetra acetate (LTA)<sup>1</sup> was found to be enhanced by the addition of pyridine or acetate ions<sup>2</sup>. The oxidation of hydroxy acids by LTA has formed the subject of recent investigations<sup>3,4</sup>. We present here the results of our studies on the effect of added bases and complexing agents on the rate of these reactions. Lactic acid has been chosen as the typical substrate. The oxidation of lactic acid by LTA, is of a total second order being first order with respect to the substrate and LTA. The reaction is profoundly susceptible to the influence of added salts especially the acetates (Table I).

TABLE I

*Effect of added salts on the rate of oxidation of lactic acid by lead tetra acetate*

Solvent: 80%HOAc—20%H<sub>2</sub>O (v/v) Temperature: 30° C

| Added salt         | [salt]<br>M | $k_2 \times 10^3$<br>lit. mol <sup>-1</sup> sec <sup>-1</sup> |
|--------------------|-------------|---|
| ..                 | ..          | 8.22  |
| NaNO <sub>3</sub>  | 0.02        | 9.62  |
|                    | 0.10        | 11.5  |
| NaClO <sub>4</sub> | 0.02        | 9.32  |
|                    | 0.10        | 10.2  |
| NaOAc              | 0.02        | 26.3  |
|                    | 0.04        | 42.8  |
|                    | 0.06        | 58.8  |
|                    | 0.08        | 71.9  |
|                    | 0.10        | 89.6  |

Pyridine, quinoline, 2, 2'-dipyridyl and 1, 10-phenanthroline when used as complexing agents/bases bring about considerable rate acceleration (Table II). In fact, there seems to be a linear dependence between the rate and the concentration of the added reagent. Separate experiments show that under the reaction conditions employed, pyridine, quinoline, 2, 2'-dipyridyl and 1, 10-phenanthroline were not oxidised by LTA in any significant measure,

It is to be noted that the bidentate ligands 2,2'-dipyridyl and 1, 10-phenanthroline have a more pronounced effect than pyridine and quinoline for a given concentration.

TABLE II

*Effect of added complexing agents/bases on the rate of oxidation of Lactic acid by lead tetra acetate*

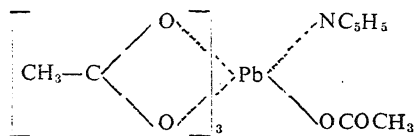
Solvent: 90%HOAc—10%H<sub>2</sub>O (v/v)

Temperature: 30° C

| Added substance     | (added substance)<br>M $\times 10^4$ | $k_2 \times 10^3$<br>lit. mol <sup>-1</sup> sec <sup>-1</sup> |
|---------------------|--------------------------------------|---|
| ..                  | ..                                   | 2.31  |
| Pyridine            | 44.84                                | 4.68  |
|                     | 89.69                                | 6.98  |
|                     | 124.7                                | 8.96  |
|                     | 179.4                                | 10.9  |
|                     | 249.4                                | 14.2  |
|                     | 498.8                                | 24.2  |
| Quinoline           | 47.98                                | 6.06  |
|                     | 95.96                                | 10.8  |
|                     | 143.9                                | 12.5  |
| 2,2'-Dipyridyl      | 39.56                                | 20.2  |
|                     | 79.43                                | 41.5  |
|                     | 118.7                                | 60.3  |
|                     | 158.2                                | 73.1  |
|                     | 197.8                                | 86.3  |
|                     | 317.7                                | 120   |
| 1,10 phenanthroline | 40.08                                | 58.5  |
|                     | 80.15                                | 106   |
|                     | 160.1                                | 177   |
|                     | 240.2                                | 228   |
|                     | 320.3                                | 356   |

A mechanism involving the formation of a Pb (IV)-hydroxy acid monoester (by a nucleophilic displacement of an acetoxy group by the hydroxyl function of the acid), followed by the rate determining decomposition of the monoester, to products has been proposed for this reaction. Alternatively, the mono acetoxy ester could, in a slow step interact with an adjacent—COOH group, to give a cyclic ester which could then decompose to give the products. It is highly probable that the added complexing reagents are functioning, more as bases catalysing the reaction, rather than as complexing ligands. For, in the proposed mechanism, an added

base can facilitate a proton removal either in the slow decomposition of the monoester or aid the formation of the cyclic ester. Base catalysis in the LTA oxidation<sup>5-6</sup>, of 1, 2-diols and formic acid lends further credence to this view. It is also possible that pyridine displaces the acetate, to generate a more reactive Pb (IV) compound<sup>2</sup>. A lead tetraacetate pyridinate has actually been isolated<sup>7</sup> and is reported to have the structure.



This has been shown to have a greater oxidising power than LTA itself. Complexes of this type can be envisaged for the other ligands. In the case of 2, 2'-dipyridyl and 1, 10-phenanthroline, a first order dependence on [ligand] is observed. This is not unexpected as 2, 2'-dipyridyl and 1, 10-phenanthroline are better complexing agents than pyridine or quinoline.

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### METAL CHELATES OF $\alpha$ -BROMOACETO- ACETANILIDE

AMONG the metal chelates of  $\beta$ -dicarbonyl compounds, only metal acetylacetonates have received adequate attention<sup>1</sup>. Alpha substituents like nitro and diazonium, which are unstable on acetylacetonates, are stabilised in metal acetylacetonates. Synthetic advantage has also been derived through these substituted acetylacetonates<sup>2-3</sup>. Such possibilities prompted an extension of these studies to other  $\alpha$ -substituted  $\beta$ -dicarbonyl compounds. Hence this investigation on  $\alpha$ -bromoacetoacetanilide and its metal chelates.

The ligand<sup>4</sup> was prepared by adding slowly an aqueous-methanolic (1:1 by volume) solution of bromine (4%) to a stirred solution of acetoacetanilide (4 g) in normal sodium hydroxide solution (about 25 ml) until the colour of bromine persisted even after long stirring. The resulting residue was filtered, washed with water and then with a little acetone, and sucked dry.

Among the metal chelates prepared (through slight modifications of the methods generally employed for the syntheses of  $\beta$ -dicarbonyl complexes<sup>5-7</sup>), those which have been obtained pure (judged from their analytical data for metal and nitrogen) are listed in Table I.

The complexes have been further characterised on the basis of their 'Nujol mull' infra-red spectra (recorded in the 4000-650  $\text{cm}^{-1}$  region on a Perkin-Elmer 257 spectrometer). The ligand shows its  $\nu$  N-H around 3200  $\text{cm}^{-1}$ , which in the complexes is shifted to about 3350  $\text{cm}^{-1}$ , evidently due to disengagement of the N-H from intramolecular hydrogen bonding<sup>8</sup>. This shift is also construed as an evidence for the abstention of the nitrogen atom from co-ordinating to the metal, since otherwise only a depression of the N-H stretching frequency could have resulted<sup>9</sup>.

The free ligand shows absorptions at 1730 and 1650  $\text{cm}^{-1}$  due to the ketone and amide carbonyl groups respectively (*c.f.* assignments of the spectral bands of acetoacetanilide<sup>6</sup>). In the chromium complex these are respectively at 1560 and 1585  $\text{cm}^{-1}$ , showing that both the carbonyl oxygens are bonded to the metal as in metal  $\beta$ -keto-enolates<sup>5,6,10</sup>, as shown in Fig. 1. From spectral similarity it is evident that the other complexes of Table I are also of the same inner complex type-

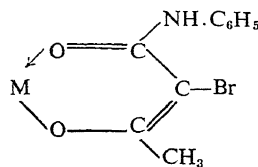


FIG. 1. Illustration of the mode of chelation.

However the amide carbonyl frequency (amide I) is much higher in the less stable complexes than in the chromium complex; this frequency is about the same in a few complexes as in the free ligand. At least, in part, this could be due to the electron attracting  $\alpha$ -substituent, asserting its role when the metal-ligand bond is weak. Electron withdrawal by substituents is known to raise amide I frequency<sup>11</sup>. In order to gain further insight into the causes of the amide I frequency shifts in these complexes, an investigation

TABLE I

| Sl. No. | Compound                             | Solvent from which recrystallised | Yield (%) | Colour          | m.p. (°C)    | Analytical data† |             |
|---------|--------------------------------------|-----------------------------------|-----------|-----------------|--------------|------------------|-------------|
|         |                                      |                                   |           |                 |              | Metal %          | Nitrogen %  |
| 1       | $\alpha$ -bromoacetoacetanilide (LH) | Ethyl alcohol                     | 80        | Colourless      | 135–137      | ..               | ..          |
| 2       | [BeL <sub>2</sub> ]                  | .. do.                            | 49        | do.             | 202          | 1.65 (1.73)      | 5.32 (5.39) |
| 3       | [CuL <sub>2</sub> ]                  | .. Acetone                        | 61        | Pale green      | *            | 10.95 (11.08)    | 5.03 (4.88) |
| 4       | [NiL <sub>2</sub> ]                  | .. do.                            | 52        | Pale yellow     | about 185 d. | 9.98 (10.32)     | 4.87 (4.92) |
| 5       | [VOL <sub>2</sub> ]                  | .. †                              | 30        | Bluish green    | *            | 8.79 (8.84)      | 4.80 (4.85) |
| 6       | [MnL <sub>2</sub> ]                  | .. †                              | 54        | Brownish black  | *            | 6.73 (6.69)      | 5.01 (5.12) |
| 7       | [CrL <sub>2</sub> ]                  | .. Ethyl alcohol                  | 80        | Yellowish green | about 155 d. | 6.32 (6.36)      | 5.22 (5.14) |

d. Decomposes. \* Not melting or visibly decomposing up to 360° C. † Insoluble in common organic solvents, hence only washed repeatedly with warm solvents. ‡ Percentages found are noted first followed by calculated percentages in brackets.

on other  $\alpha$ -substituted- $\beta$ -ketoanilides has been initiated.

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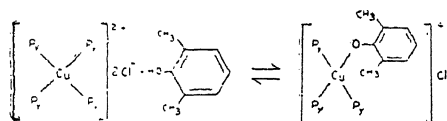
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### LIGAND EXCHANGE IN POLYMERIZATION CATALYSIS

OFTEN, to explain the results of polymerizations catalysed by metal complexes, substitution of a ligand by a monomer molecule in the complex is suggested without sufficient proof. To explain a second-order dependence of the rate of acrylamide

polymerization on monomer concentration with aquopentamminecobalt (III) complex as the initiator, Delzenne<sup>1</sup> suggested participation of the monomer in the formation of the initiating radicals through a reversible substitution of the coordinatively bonded water molecule by acrylamide (analogous to the substitution of a ligand by N-methylacetamide<sup>2</sup> in [Co(en)<sub>3</sub>X<sub>2</sub>]X followed by photochemically induced electron transfer within the intermediate complex. We considered<sup>3,4</sup> the analogy to be inappropriate, because while there was a distinct change of colour on mixing N-methylacetamide and solution of the complex and the shifts in  $\lambda_{\max}$  values were also reported, in the case of the aquopentamminecobalt (III) complex no such colour change or change in optical density measurements were reported. Delzenne concurred with us in a subsequent private communication. In the case of diazidotetramminecobalt (III) azide also there was no significant change in the spectrum of the complex on addition of acrylamide proving that no complexation by substitution was involved<sup>3</sup>.

Recently Komoto and Ohmura<sup>5</sup> studying the oxidative polymerization of 2,6-xyleneol by amine complexes have postulated ligand exchange reaction between the copper (II) pyridine complex and the monomer which then causes initiation. In the



light of the foregoing considerations we considered such a reaction to be unlikely. The spectrum of a 0.05 M solution of the copper (II) pyridine complex was recorded in a Beckman DU 2 model Spectrophotometer (*vide* Fig. 1). Absorbance was



negligible in the region 380–500 nm, but increased to a maximum at 760 nm. The spectrum of the copper pyridine complex-2, 6-xyleneol mixture was practically the same, with the xyleneol alone showing decreasing absorbance at increasing wavelengths.

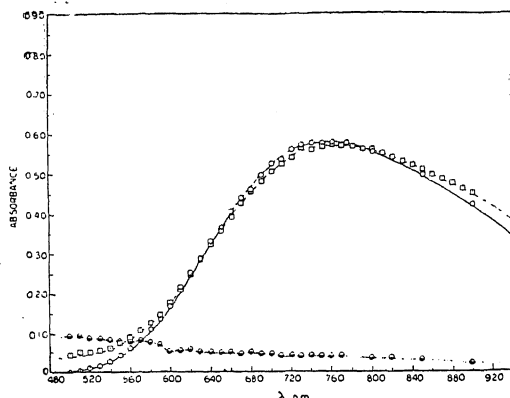


FIG. 1. Absorption spectrum of : ○ — copper pyridine complex ; □ --- copper pyridine complex-2, 6-xyleneol mixture ; ● .... 2, 6-xyleneol.

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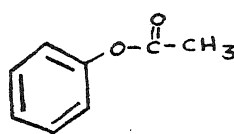
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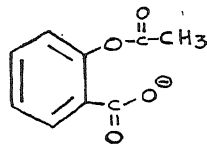
#### FIELD EFFECTS *VIS-A-VIS* SOLVENT EFFECTS IN THE ALKALINE HYDROLYSIS OF PHENYL ACETATE AND *o*-CARBOXYPHENYL ACETATE

In the alkaline hydrolysis of a series of dicarboxylic esters in aqueous DMSO and aqueous EtOH, we had observed a considerable increase in the  $k_I/k_{II}$  ratio in aqueous DMSO compared to aqueous EtOH<sup>1,2</sup>. We have now studied the alkaline hydrolysis of phenyl acetate (I) and *o*-carboxyphenyl acetate (II) in aqueous DMSO and aqueous EtOH with a view to finding out the effect of solvent on such a repulsion between an inbuilt carboxylate anion and OH<sup>-</sup> ion on the rate of saponification. The essential difference between these systems and the dicarboxylic esters previously studied is that in

the present case the inbuilt carboxylate group is in the alkyl portion of the ester.



I



II

Spectrophotometric methods were employed to follow the kinetics of the reaction. The rate constants (obtained in triplicate) were evaluated from semilogarithmic plots of  $(A_\infty - A_t)$  versus  $t$ . The kinetic results are presented in Table I. The second order rate constants were obtained from the pseudo first order rate constants. Table II provides a comparative rate picture in the various solvent mixtures.

TABLE I

Rate constants for the saponification of phenyl acetate (I) and *o*-carboxyphenyl acetate (II) at 15.5° C in aqueous solvent mixtures (v/v)

| Compound | Solvent  | $10^3 k_{obs}$<br>(sec <sup>-1</sup> ) | (NaOH)<br>M | $K_{OH}$ (lit.<br>mole <sup>-1</sup><br>sec <sup>-1</sup> ) |
|----------|----------|--|-------------|---|
| I        | 50% EtOH | 91.6                                   | 0.1         | 0.916   |
| I        | 80% EtOH | 73.7                                   | 0.1         | 0.737   |
| I        | 80% DMSO | 68.2                                   | 0.01        | 6.960   |
| II       | 50% EtOH | 9.41                                   | 0.1         | 0.094   |
| II       | 80% EtOH | 9.67                                   | 0.1         | 0.098   |
| II       | 80% DMSO | 49.70                                  | 0.1         | 0.497   |

TABLE II

$k_I/k_{II}$  values in mixed solvents\*

| Solvent  | $k_I/k_{II}$ |
|----------|--------------|
| 50% EtOH | 9.7          |
| 80% EtOH | 7.4          |
| 80% DMSO | 14.0         |

\* Data from Table 1.

The general expectation of the rate enhancement in aqueous DMSO has been realised and this is naturally due to the cumulative effect of two factors : (i) poorly solvated and therefore highly reactive hydroxide ion and (ii) the increased transition state solvation in DMSO<sup>3</sup>.

The interesting finding of this investigation is the increase in the ( $k/k_{11}$ ) ratio in DMSO compared to EtOH. The decreased reactivity of *o*-carboxyphenyl acetate over phenyl acetate is due to the electrostatic repulsion between the attacking OH<sup>-</sup> ion and the carboxylate group. This is obviously much more pronounced in DMSO because of the presence of the poorly solvated hydroxide ion in aqueous DMSO. This leads to lower  $k_{11}$  values and hence the higher  $k_1/k_{11}$  ratio in aqueous DMSO. This behaviour is analogous to the one noticed in the alkaline hydrolysis of dicarboxylic esters. The operation of such a rate retarding factor is further evident if one compares this ratio in 50% EtOH and 80% EtOH. The decrease in the ratio with decrease in the dielectric constant is also in the expected direction. The decreased dielectric constant will result in the diminution of the field effect of the carboxylate anion on the reaction centre. Increased ion-pair formation is another consequence leading to a drop in the effective concentration of the carboxylate anion.

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#### EFFECT OF HYDROGEN PEROXIDE ON BUFFALO MILK PROTEIN FRACTIONS

THE nutritional aspects of H<sub>2</sub>O<sub>2</sub> treated casein, whey, peptide and free amino acids, have been investigated in this laboratory<sup>1</sup>. In this paper, chromatographic studies on the composition of casein (several samples) and H<sub>2</sub>O<sub>2</sub> treated casein are compared with the electrophoretic patterns.

Buffalo casein was isolated by isoelectric precipitation<sup>2</sup>. 100 mg each of the untreated and the H<sub>2</sub>O<sub>2</sub> treated samples were hydrolysed by 6 N HCl at 120°C for 24 hours. After evaporation, the hydrolysates were made upto 10 ml in 10% isopropanol. The hydrolysates were then spotted on Whatman No. 1 filter paper and irrigated with *n*-butanol, water acetic acid (4 : 1 : 1) by employing ascending paper chromatographic technique<sup>3</sup>. The separated amino acids were identified by treatment with ninhydrin in 95% aq. acetone. Treated and untreated caseins were separately dissolved in alkali and the protein fractions were separated by both paper electrophoresis<sup>4</sup> and starch-gel electro-

phoresis<sup>5,6</sup>. The H<sub>2</sub>O<sub>2</sub> treated and the untreated whey samples were also subjected to electrophoretic separation using starch-gel.

**Results and Discussion.**—From the Chromatogram in Fig. 1, it is clear that the amino acid tyrosine is readily destroyed by the addition of

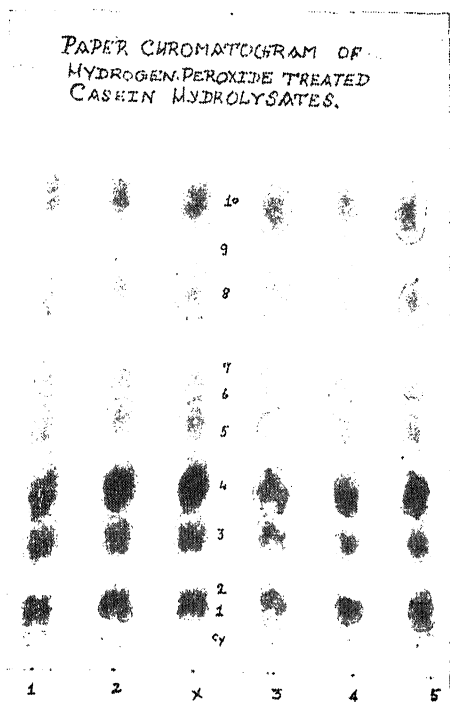


Fig. 1. 1. 0.02% (w/v) of H<sub>2</sub>O<sub>2</sub> treated casein hydrolysate. 2. 0.04% (w/v) of H<sub>2</sub>O<sub>2</sub> treated casein hydrolysate. X—untreated casein hydrolysate. 3. 0.06% (w/v) of H<sub>2</sub>O<sub>2</sub> treated casein hydrolysate. 4. 0.08% (w/v) of H<sub>2</sub>O<sub>2</sub> treated casein hydrolysate. 5. 0.10% (w/v) of H<sub>2</sub>O<sub>2</sub> treated casein hydrolysate.

Cy : Cystine band (1) Lysine, Histidine bands, (2) Arginine band, (3) Serine, Glycine, and Aspartic acid bands. (4) Glutamic acid and Threonine bands. (5) Alanine band. (6) Proline band. (7) Tyrosine band. (8) Valine and Methionine bands. (9) Phenyl alanine bands. (10) Leucine and Iso-leucine bands.

0.10% H<sub>2</sub>O<sub>2</sub> (w/v) to milk. Sulphur containing amino acids are also readily affected by the gradual increase in the conc. of H<sub>2</sub>O<sub>2</sub>. Cystine bands get diffused in the chromatogram. Leucine and Iso-leucine are very much affected at high concentrations of H<sub>2</sub>O<sub>2</sub>. The Chromatogram in Fig. 1 also indicates that the amino acid composition of casein is not affected by the addition of H<sub>2</sub>O<sub>2</sub> upto 0.10% (w/v). Higher concentrations, however,

amino acids and thus reduce the nitrogen value of milk.

Electrophoretic studies as shown by starch-gel electrophoresis (Fig. 2) indicate that  $\kappa$ -casein is least affected by the addition of  $H_2O_2$  and beta-casein is moderately affected, whereas alpha-casein remains unaffected. The electrophoretic pattern of whey protein (Fig. 3) shows only two bands in starch-gel electrophoresis. Lactoglobulin is more heavily affected than lactalbumin<sup>7</sup>.

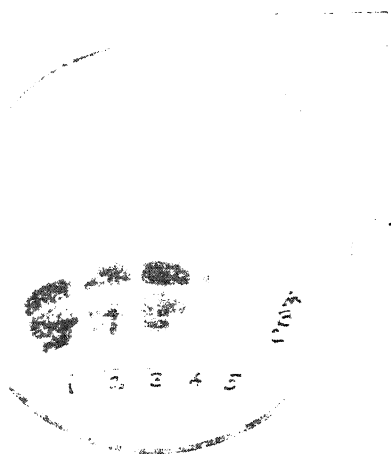


FIG. 2. Buffalo casein fraction. 1. 0.02% (w/v) of  $H_2O_2$  treated casein. 2. 0.04% (w/v) of  $H_2O_2$  treated casein. 3. Untreated casein. 4. 0.08% (w/v) of  $H_2O_2$  treated casein. 5. 0.10% (w/v) of  $H_2O_2$  treated casein.

A—Alpha-casein fraction. B—Beta-casein fraction. C— $\kappa$ -casein fraction.

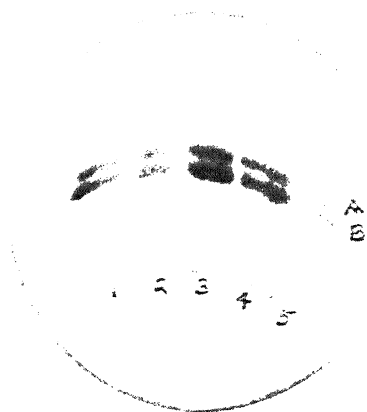


FIG. 3. Buffalo whey fraction. 4. 0.02% (w/v) of  $H_2O_2$  treated whey. 1. 0.04% (w/v) of  $H_2O_2$  treated whey. 3. Untreated whey. 2. 0.08% (w/v) of  $H_2O_2$  treated whey. 5. 0.10% (w/v) of  $H_2O_2$  treated whey. A—Lactoglobulin; B—Lactalbumin.

In paper electrophoresis of buffalo whey, three protein fractions were separated, and one of the fractions was found to increase with the increasing conc. of  $H_2O_2$ . Further investigation on this separation is in progress.

The authors thank the authorities of Loyola College, Madras, for the facilities, Dr. N. C. Ganguly of N.D.R.I. (Karnal) for starch-gel electrophoretic separation and Rev. Fr. Sebastian Kalarickal, S.J., for his interest and encouragement.

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### CHROMATOGRAPHIC SEPARATION OF Cu (II), Ni(II) AND Zn (II) IN PRESENCE OF COMPLEXING AGENTS

The presence of complexing agents along with metal ions, affect the diffusion of ions through filter paper<sup>1,2</sup>. In the present paper a rapid quantitative separation of  $Cu^{+2}$ ,  $Ni^{+2}$  and  $Zn^{+2}$  has been described in presence of complexing agents, using ascending filter paper chromatographic technique.

Chromatograms were run in glass jar (15 cm x 12 cm) having a height of 30 cm. Strips of Whatman filter paper No. 1 (3 cm x 32 cm) were used. A series of solvent systems was tried out, of which, the mixed solvent containing 90% acetone + 6% conc. HCl + 4% water (v/v) proved to be the most successful. For locating the spots, the strip was first exposed to ammonia vapour and then was sprayed with 0.1% Rubeanic acid in alcohol. Copper appears as a green and nickel as a blue spot. 0.5% Dithiazone in chloroform gave a pink spot for zinc.

The effect of the complexing agents, oxalate, tartarate and citrate was tried on the separation of Cu (II), Ni (II) and Zn (II) in three different ways. (a) by adding the complexing agents to the solvent, (b) by using filter paper impregnated with complexing agents and (c) by adding complexing

TABLE I  
Experiments on quantitative separation

| Conditions of Study   | Time of Run | Experimental value ( $\mu\text{g}$ ) |                  |                  | Theoretical value ( $\mu\text{g}$ ) |                  |                  |
|---|-------------|--------------------------------------|------------------|------------------|-------------------------------------|------------------|------------------|
|   |             | Zn <sup>2+</sup>                     | Cu <sup>2+</sup> | Ni <sup>2+</sup> | Zn <sup>2+</sup>                    | Cu <sup>2+</sup> | Ni <sup>2+</sup> |
| (i) Citrate added to metal solution in 1:2 (metal: citrate molal ratio) | 30 mts.     | 41.0                                 | 120.0            | 125.0            | 40.0                                | 123.0            | 130.0            |
| (ii) do.  | 1 hour      | 45.0                                 | 110.0            | 130.0            | 40.0                                | 123.0            | 130.0            |
| (iii) Tartrate added to metal solution                                  | 1 hour      | 70.0                                 | 215.0            | 234.0            | 81.0                                | 246.0            | 260.0            |

agents to the metal salt solutions. The most significant result is obtained when twice the excess of the citrate is added to the metal salt solution ( $R_F$  values Zn<sup>2+</sup> = 1.0; Cu<sup>2+</sup> = 0.69; Ni<sup>2+</sup> = 0.09). Good results are also obtained when tartrate is added ( $R_F$  values Zn<sup>2+</sup> = 0.96; Cu<sup>2+</sup> = 0.54; Ni<sup>2+</sup> = 0.08) to the metal solutions.

**Quantitative Separation and Estimation of Ni<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>.**—Two chromatograms were run under similar conditions. One of these chromatograms was developed and the position of the spots located. The undeveloped chromatograms were cut into three portions depending on the position of individual components which were then extracted from the filter paper into solution and estimated spectrophotometrically.

**Cu<sup>2+</sup> :** Copper at pH 8.5 (in presence of citric acid) was extracted with lead diethyl dithiocarbamate in chloroform and the absorbance noted at 560 nm.

**Ni<sup>2+</sup> :** Pink complex of Ni(DMG)<sub>2</sub> was extracted in chloroform at pH 7.5 and the absorbance noted at 420 nm.

**Zn<sup>2+</sup> :** Estimated using xylenol orange at pH 6.3 (acetic acid, sodium acetate buffer) and absorbance was noted at 570 nm.

The absorbance of unknown solution is compared from the calibration curve obtained from standard known solutions under similar conditions. Some of the results are summarized in Table I.

Thanks are due to Prof. R. H. Sabasrabudhey for providing facilities.

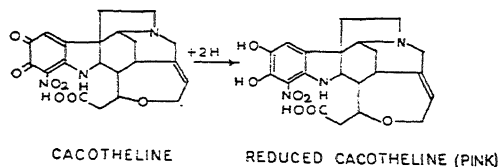
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Nagpur, December 8, 1974.

## DETECTION OF PYROGALLOL WITH CACOTHELINE

MENTION may be made of the reagents like HCHO-HCl<sup>1</sup> and tetramethyl aminobenzophenone<sup>2</sup> for the sensitive detection of pyrogallol. In this communication the details of the detection of pyrogallol with cacotheline are given. The present method is simple, comparatively more selective and sensitive over the other methods.

**Experimental procedure.**—0.4 ml of conc. sulphuric acid (AR) is mixed with 0.05 ml of 0.005% cacotheline and 0.05 ml of test solution. A stable pink colour is produced indicating the presence of pyrogallol (limit of identification: 25  $\mu\text{g}$  ml). The sensitivity is nearly doubled by increasing the temperature to 70° C. It is found that phenol, hydroquinone, *m*-cresol, *p*-cresol, pyrocatechol, 1-naphthol, 2-naphthol, gallic acid, phloroglucinol and hydroxyhydroquinone will not interfere in 3 fold excess. Resorcinol and *o*-cresol will interfere because of the brown colour.

The pink compound was found to be the reduced product of cacotheline as given below and the spectrum exhibits maximum absorption in the region of 525-530 nm<sup>3</sup>.



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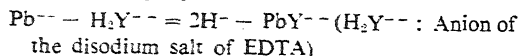
### ESTIMATION OF OXALATE IONS AND OXALIC ACID USING EDTA

The estimation of the oxalate anion and oxalic acid by the EDTA method involves the quantitative precipitation of the oxalate by adding a known excess of standard lead acetate solution and back titrating the excess lead acetate with EDTA. The method is simple and accurate. The precipitation merely involves mixing the cold solutions and the easily filterable precipitate shows practically no creep.

**Discussion.**—The method adopted is the converse of the usual method for the estimation of lead<sup>2+</sup>. It is found that under the conditions of precipitation with excess of lead acetate, oxalic acid and oxalate are both quantitatively precipitated and no distinction in practical procedure is called for. The results both in the case of oxalic acid and oxalate show practically the same accuracy, ~ 0.5%. The presence of small quantities of oxidisable impurities might pose a serious problem in the case of permanganimetry, whereas the accuracy of EDTA method is not affected by such impurities.

Lead reacts with Erio T in ammoniacal medium. However, at this pH the lead is precipitated as hydroxide. To avoid precipitation, the addition of tartrate ion is necessary. The lead-tartrate complex is sufficiently stable to keep the lead in solution, but it is not sufficiently so to prevent its reaction with the indicator and with EDTA. The colour intensity of the lead-Erio T complex is somewhat dependent upon the concentration of the tartrate called "auxiliary complex-former" and too large an excess of the auxiliary complex-former should be avoided. The colour of the indicator before the end point is a bluish violet. However the disappearance of the last reddish tint is very abrupt and takes place within the range of a drop or part of a drop of titrant.

The following equation is relevant :



For comparing the EDTA method with the conventional method, the following procedure is adopted. Analar grade oxalic acid or sodium oxalate is used for preparing the solutions. A known excess of a standard solution of Pb<sup>++</sup> ion is added to an aliquot of the oxalate solution, the precipitate is filtered off and the filtrate titrated against EDTA solution of known strength. The strength of the oxalate solution is then calculated and compared with its known value. Similarly a known excess of oxalate solution is added to a known volume of lead salt solution and after filtering off the lead oxalate, the solution is titrated

against permanganate solution independently standardised. The known strengths of the permanganate and the lead salt solutions are then used to calculate the concentration of the oxalate solution. In the titration of Pb<sup>++</sup> ion against EDTA, the pH is kept at 10 by buffering with tartrate-ammonia. Erio T is used as the indicator and the end point is the colour change from violet to blue. The results are excellent and the accuracy is usually better than 0.6%.

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### GROUP IB METAL BISTHIOSALICYLATO MERCURATES

DISODIUM bisthiosalicylato mercurate was prepared as reported earlier<sup>3</sup>. Copper and silver bisthiosalicylato mercurates were obtained by adding varying amounts of 0.01 M disodium bisthiosalicylato mercurate solution to fixed quantities of 0.01 M copper and silver sulphate solutions obtained from BDH (India) A.R. quality samples. Copper yielded a dark-green thick precipitate and a curdy light yellow precipitate was obtained in the case of silver bisthiosalicylato mercurate. The reaction mixtures were kept overnight at room temperature (25° C). The precipitates were filtered, washed with distilled water and dried in oven at 100° C for two hours. 0.01 M cold solution prepared from chloroauric acid obtained from Johnson Mathey Co., Ltd. (London), was standardized for its Au<sup>3+</sup> content by the thiosalt method<sup>4</sup>. The pH of the solution was raised to 5.8. A fixed quantity of 0.01 M disodium bisthiosalicylato mercurate solution was treated with varying quantities of 0.01 M Au<sup>3+</sup> solution. During the precipitation, the pH of the solutions goes on decreasing. Gold bisthiosalicylato mercurate when fresh is dark-yellow in colour but becomes black after drying in oven.

Gold mercurate decomposes when dried at 100° C, whereas copper and silver bisthiosalicylato mercurates do not decompose at this temperature.

Copper and silver bisthiosalicylato mercurates were analysed for sulphur and mercury contents. Sulphur was estimated by fusing with fusion mixture and then completing estimation gravimetrically<sup>5</sup> as

BaSO<sub>4</sub>. Mercury was estimated quantitatively as HgS by thioisalt method<sup>4</sup>. The method was slightly modified as the precipitates were filtered from the warm solution and washed thoroughly with ethanol to remove thiosalicylic acid which coprecipitates along with HgS. The experimental values of mercury and sulphur corresponded well with the theoretical values.

The infrared spectra of the solid metal bithiosalicylato mercurates were recorded between 600–4000 cm<sup>-1</sup> using nujol mulls on Perkin-Elmer 337-Spectrophotometer. The i.r. spectrum of thiosalicylic acid shows bands corresponding to —SH, C=O and C—S stretching frequencies appearing at 2250, 1700 and 755 cm<sup>-1</sup>, respectively. The comparison with the nujol mull shows that bands corresponding to —SH frequencies disappear completely. But the bands corresponding to C=O stretching vibrations shift to lower frequency and are observed to split. C—S stretching vibrational bands also shift to a lower frequency. The disappearance of —SH frequency band and the lowering of C—S stretching frequency indicate the coordination through sulphur atom. The coordination through carboxyl oxygen and carboxylic group is indicated by lowering of C=O stretching frequencies. The splitting of the band may be due to coupling of some vibrations.

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#### EXCYSTMENT OF *SCHIZOPYRENUS RUSSELLI* BY INDIVIDUAL AMINO ACIDS AND THEIR MIXTURES

SINGH, Mathew and Anand<sup>1</sup> (1958) showed that aqueous extract of *Aerobacter* sp. and *Escherichia coli* caused nearly cent per cent excystment of cysts of *Schizopyrenus russelli* at a suitable pH. It was shown that part of the excystment inducing property of *Aerobacter* sp. extract was due to

certain amino acids. Chemically pure amino acids also gave varying degrees of excystment. In general the excystment caused by individual amino acids was much lower than that caused by the bacterial extract. Drozanski<sup>2</sup> (1961) also reported that aqueous extract of *A. aerogenes* and certain amino acids were able to cause excystment of cysts of soil amoebae belonging to the family Hartmannellidae. Jefferies<sup>3</sup> (1962) found that amino acids could cause excystment of a ciliate, *Pleurotricha lanceolata*. Singh, Datta and Dutta<sup>4</sup> showed that the percentage excystment of cysts of *S. russelli* with L-isoleucine, L-arginine monohydrochloride, L-alanine, L-serine and L-glutamic acids at 2.0% concentration in distilled water, pH about 6.5, was significantly higher than at 0.25% or 0.125%. When mixtures of all the amino acids at these concentrations were used, the percentage excystment was markedly increased. Singh, Datta and Dutta<sup>4</sup> suggested that nearly cent per cent excystment of *S. russelli* obtained by *E. coli* extract may be due to a mixture of amino acids and other factors in the extract. Rastogi, Sagar and Agarwala<sup>5</sup> (1973) observed that the excystment of *S. russelli* was quicker in a mixture of amino acids containing riboflavin than that obtained in the mixture of amino acids alone. The present communication deals with excystment of *S. russelli* by individual amino acids and their mixtures.

*S. russelli* was grown on non-nutrient agar plates supplied with *E. coli*. Eight to 10 days old cysts were harvested and freed from living and dead bacteria, and used for studying the excystment according to the method reported earlier<sup>4</sup>. The chemically pure amino acids used were sterilized by autoclaving at 15 lb/sp. in. pressure for 15 min.

It has been found by Singh, Datta and Dutta<sup>4</sup> that cysts of *S. russelli*, produced at different times with *E. coli*, gave somewhat varying degrees of excystment with L-isoleucine, L-arginine monohydrochloride, L-alanine, L-serine and L-glutamic acid. Therefore cysts prepared from the same batch were employed by them to show that mixture of the above amino acids gave higher percentage excystment than that obtained by individual amino acids. In these experiments the concentration of amino acids used in the mixture was five times more than that in the case of individual amino acids. In order to show the additive effect of mixture of the amino acids used by Singh, Datta and Dutta<sup>4</sup>, cysts of *S. russelli* prepared from the same batch were used and the concentration of amino acids in the mixture was kept the same as in the case of individual amino acids. The results presented in Table I show that the percentage excystment with mixture of amino

TABLE I

*Additive effect of mixture of five amino acids on the excystment of S. russelli cysts*

| Amino acids  | pH  | Total amino acid concentration |     |    |      |     |    |       |      |    |        |     |    |
|--|-----|--------------------------------|-----|----|------|-----|----|-------|------|----|--------|-----|----|
|  |     | 1%                             |     |    | 0.5% |     |    | 0.25% |      |    | 0.125% |     |    |
|  |     | A                              | B   | C  | A    | B   | C  | A     | B    | C  | A      | B   | C  |
| L-Glutamic acid  | 6.2 | 171                            | 86  | 50 | 119  | 48  | 40 | 132   | 48   | 36 | 146    | 49  | 33 |
| L-Arginine monohydrochloride                                 | 6.2 | 125                            | 68  | 54 | 129  | 48  | 37 | 121   | 44   | 36 | 109    | 38  | 34 |
| L-Isoleucine   | 6.2 | 151                            | 80  | 52 | 118  | 49  | 41 | 131   | 52   | 39 | 180    | 46  | 25 |
| L-Alanine  | 6.2 | 570                            | 370 | 64 | 64   | 41  | 64 | 206   | 121  | 58 | 133    | 67  | 50 |
| L-Serine   | 6.2 | 37                             | 19  | 51 | 72   | 42  | 58 | 166   | 58   | 35 | 139    | 47  | 34 |
| Mixture containing equal amount of all the above amino acids | 6.2 | 246                            | 211 | 85 | 424  | 346 | 81 | 1462  | 1218 | 83 | 1191   | 933 | 78 |
| Distilled water control                                      | 5.8 | 146                            | 0   | 0  |      |     |    |       |      |    |        |     |    |
| <i>E. coli</i> extract                                       | 6.5 | 164                            | 148 | 90 |      |     |    |       |      |    |        |     |    |

A=No. of cysts; B=No. excysted; C=Percentage excystment.

TABLE II

*Additive effect of mixture of ten amino acids on the excystment of S. russelli cysts*

| Amino acids  | pH  | Total amino acid concentration |     |    |       |     |    |        |     |    |        |     |    |
|--|-----|--------------------------------|-----|----|-------|-----|----|--------|-----|----|--------|-----|----|
|  |     | 0.5%                           |     |    | 0.25% |     |    | 0.125% |     |    | 0.031% |     |    |
|  |     | A                              | B   | C  | A     | B   | C  | A      | B   | C  | A      | B   | C  |
| L-Glutamic acid  | 6.5 | 133                            | 58  | 43 | 70    | 25  | 36 | 120    | 42  | 35 | 126    | 31  | 24 |
| L-Aspartic acid  | 6.4 | 99                             | 40  | 40 | 99    | 36  | 36 | 91     | 30  | 33 | 88     | 41  | 46 |
| L-Arginine monohydrochloride                                 | 6.4 | 129                            | 48  | 37 | 121   | 44  | 36 | 109    | 38  | 34 | 100    | 31  | 31 |
| L-Histidine  | 6.5 | 150                            | 60  | 40 | 137   | 50  | 36 | 110    | 35  | 31 | 182    | 58  | 31 |
| L-Isoleucine   | 6.5 | 79                             | 42  | 53 | 74    | 29  | 39 | 64     | 17  | 27 | 99     | 23  | 23 |
| DL-Valine  | 6.5 | 137                            | 57  | 41 | 130   | 46  | 35 | 130    | 42  | 32 | 182    | 33  | 18 |
| DL-Alanine   | 6.5 | 64                             | 41  | 64 | 32    | 20  | 63 | 157    | 50  | 31 | 169    | 85  | 40 |
| DL-Methionine  | 6.5 | 134                            | 46  | 34 | 113   | 31  | 27 | 128    | 26  | 20 | 159    | 66  | 33 |
| DL-Serine  | 6.5 | 132                            | 60  | 45 | 134   | 45  | 33 | 148    | 40  | 27 | 218    | 41  | 18 |
| DL- $\beta$ -Phenylalanine                                   | 6.5 | 109                            | 45  | 40 | 91    | 32  | 35 | 93     | 28  | 30 | 190    | 6   | 3  |
| Mixture containing equal amount of all the above amino acids | 6.5 | 76                             | 65  | 86 | 173   | 155 | 83 | 490    | 436 | 88 | 404    | 312 | 77 |
| Distilled water control                                      | 5.8 | 200                            | 0   | 0  |       |     |    |        |     |    |        |     |    |
| <i>E. coli</i> extract                                       | 6.4 | 185                            | 171 | 92 |       |     |    |        |     |    |        |     |    |

A=No. of cysts; B=No. excysted; C=Percentage excystment.

acids was much higher than that obtained with individual amino acids. It is interesting to note that the percentage excystment with the mixture of amino acids is more or less the same irrespective of the concentration of amino acids used in the mixture.

Singh, Marhew and Anand<sup>1</sup> and Singh, Datta and Dutta<sup>2</sup> have reported that ten amino acids included in Table II gave fairly good excystment of cysts of *S. russelli* at 2.0% (wt./vol.) concentration at a suitable pH. It was considered of interest to find

out whether mixture of these amino acids in very low concentrations showed any additive effect on the excystment of cysts of *S. russelli*. The results in Table II show that irrespective of the concentration of amino acids in the mixture, the percentage excystment was always significantly higher than in the case of individual amino acids.

The above findings clearly show that very low concentration of amino acids in mixture give nearly as good excystment as that obtained by aqueous extract of *E. coli* (Tables I and II). This suggests

that very high percentage of excystment obtained by *E. coli* extract upto a dilution of 1/400 (Singh, Datta and Dutta<sup>4</sup>) is probably due to a mixture of amino acids in the extract.

The authors are thankful to Dr. Nitya Nand, Director, for his continued interest and support to this problem. Thanks are also due to Dr. B. N. Singh. for helpful suggestions. Technical assistance rendered by Shri K. L. Gulati is acknowledged. One of the authors (Miss Mahmood Jehan) is thankful to C.S.I.R. for the award of a Senior Research Fellowship.

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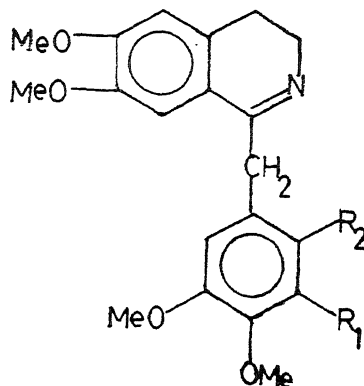
#### SYNTHESIS OF SOME HALOGENO ISOQUINOLINE DERIVATIVES

In continuation of our previous work<sup>1</sup> on the synthesis of isoquinoline derivatives we describe here the synthesis of some new halogeno-1-benzyl-3,4-dihydroisoquinoline derivatives prepared by the application of the Bischler-Napieralsky reaction on the appropriate  $\beta$ -phenethylamides. The latter were obtained by the condensation of homoveratrylamine (A) with the acid chlorides of the following acids : 3,4-dimethoxy-5-chloro-(I) 3,4-dimethoxy-5-bromo-(II) 4,5 dimethoxy-2-chloro-(II) and 4,5-dimethoxy-2-bromo-(IV)-phenylacetic acids.

The acid (I), m.p. 182–83°, was obtained by the action of alkaline hydrogen peroxide on the azalactone, m.p. 156°, prepared by heating 5-chloroveratraldehyde<sup>2</sup> with hippuric acid under the conditions of the Erlenmeyer reaction. The acid chloride from (I) prepared by heating it with thionyl chloride in dry chloroform was heated as such with (A) in dry benzene to afford the corresponding amide, m.p. 120–21°. Cyclization of the latter in the presence of  $\text{POCl}_3$  in boiling toluene yielded the required dihydroisoquinoline (V) as an oil; its picrolonate gave m.p. 243°.

The unknown acid (II), m.p. 186–87° (from water) was obtained in a similar manner<sup>3</sup> from the

azalactone, m.p. 165–66°, prepared from 5-bromoveratraldehyde<sup>4</sup>. The amide, resulting from the acid chloride of II with (A), had m.p. 128–29° (dil. alcohol). The dihydroisoquinoline (VI) crystallised from petroleum ether in colourless globules, m.p. 132°.



- |        |                   |                   |
|--------|-------------------|-------------------|
| (V)    | $R_1 = \text{Cl}$ | $R_2 = \text{H}$  |
| (VI)   | $R_1 = \text{Br}$ | $R_2 = \text{H}$  |
| (VII)  | $R_1 = \text{H}$  | $R_2 = \text{Cl}$ |
| (VIII) | $R_1 = \text{H}$  | $R_2 = \text{Br}$ |

The acid (III), m.p. 118–19°, was obtained as colourless needles from water by the direct chlorination of homoveratric acid in glacial acetic acid. Its structure was evident from its nmr spectrum in which the two aromatic protons appeared as singlets at  $\delta$  7.17 and  $\delta$  7.28. The amide isolated by the reaction of the acid chloride of (III) with (A) crystallised from alcohol, m.p. 130–32. The dihydroisoquinoline (VII) was isolated as an oil; its picrate gave m.p. 177–79°.

The acid (IV), m.p. 114–15° crystallised from water and was prepared as described by Haworth and Perkin<sup>5</sup> by the bromination of homoveratric acid in acetic acid medium. In this case also, the nmr spectrum showed the two aromatic protons as singlets at  $\delta$  7.17 and  $\delta$  7.37°. The reaction of the acid chloride of IV with (A) afforded the amide, m.p. 159–61°, which was cyclised to the dihydroisoquinoline (VIII) isolated as a picrate, m.p. 209–10°.



All the compounds gave satisfactory analysis for C, H and N. We are thankful to Shri R. S. Kulkarni for the micro analysis of the compounds.

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#### OCCURRENCE OF *LINGUATULA SERRATA* (FROLICH, 1789) IN CATTLE

THE authors while examining the mesenteric lymph glands of tuberculin positive cattle, after slaughter, came across the nymphal stages of the tongue worm, *Linguatula serrata* (Arachnida, Pentastomida, Porocephalidae) in one out of seven animals slaughtered. This lymph gland was also positive for *Mycobacteria* by direct microscopic examination using Acid-Fast technique. This aroused our curiosity and the mesenteric lymph glands of cattle and sheep slaughtered at the Corporation slaughter house, Bangalore, were examined randomly selecting 4-5 glands from each animal. Of the 42 cattle examined, 5 were harbouring nymphal stages of *L. serrata* whereas the lymph glands of the 10 sheep examined were found free from this parasite. One nymph was recovered from each gland except in one gland where 3 nymphs were recovered. Only two lymph glands, harbouring *L. serrata* collected from two different animals, were examined for *Mycobacteria* but the test proved to be negative.

The prevalence both of nymphal stages and adult forms of *L. serrata* in man and dogs, and larval and nymphal forms in buffaloes, sheep, goats, pigs, camels and an Indian python has been recently reviewed<sup>2,3,5</sup>. The only report of the occurrence of *L. serrata* as larval forms in cattle is that of Alwar<sup>1</sup>. The present finding, therefore, reports the existence of the nymphal forms of *L. serrata* in cattle of India for the first time.

Since the adults of this parasite are found to occur in the respiratory passages and the encysted

nymphs in the lymph glands and liver of man (probable aetiological agents of Halzoun and Marrara syndrome), the public health significance of this infection in domestic and wild animals is stressed. Referring to the affinity for the nymphs and *Mycobacteria* to occur concomitantly, Lapage<sup>4</sup> suspected the possible attraction of the nymphs to the tuberculous lymph glands. The present authors have also observed the occurrence of both the nymph and *Mycobacteria* in the same lymph gland. This observation enhances the suspicion raised by Lapage<sup>4</sup> and, therefore, further investigation is warranted.

The authors wish to thank Dr. S. Sujaya Kumar, Veterinary Surgeon, Corporation slaughter house, Bangalore, for his assistance during collection of materials from the slaughter house.

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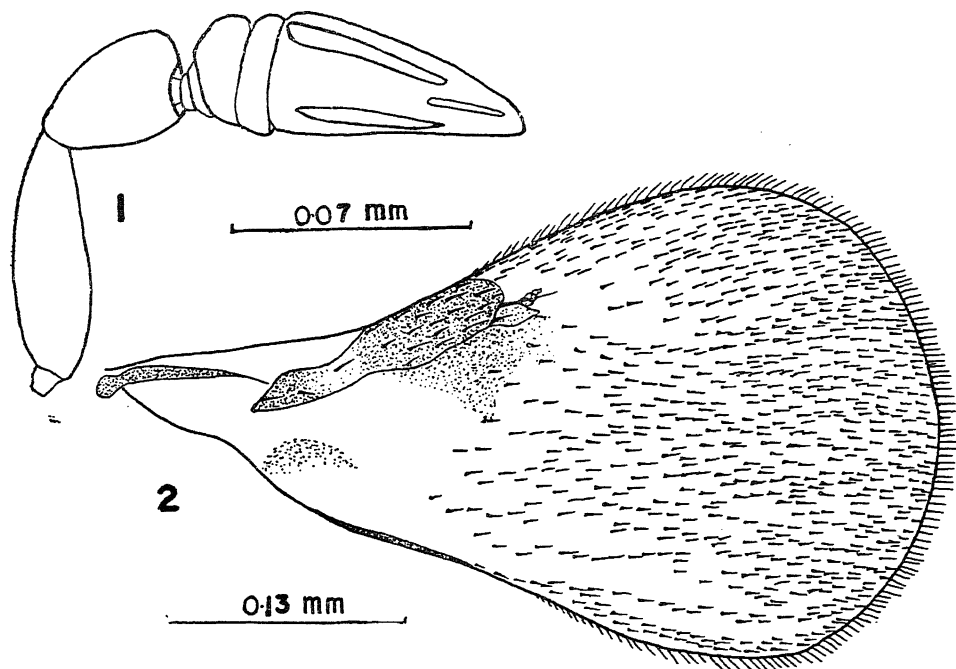
#### A NEW SPECIES OF THE GENUS *BRACHYGRAMMATELLA* GIRAULT (HYMENOPTERA: TRICHOGRAMMATIDAE) FROM ALIGARH, INDIA

THE genus *Brachygrammatella* was erected by Girault (1915) with *Brachygrammatella nebulosa* Girault as type species. Doutt and Viggiani (1968) synonymised *Pseudbrachygramma* Girault with *Brachygrammatella* Girault. However, they recognized *Pseudbrachygramma* as a subgenus of *Brachygrammatella*. The distinguishing characters of this genus have been given in detail by Doutt (1968). The genus is recorded for the first time from India. Doutt's (1968) key to subgenera and species of *Brachygrammatella* Girault has been revised to accommodate *Brachygrammatella indica* sp. n.

Revised Key to Subgenera and Species of  
*Brachygrammatella* Girault

1. Fore wings with setae immediately beneath marginal vein; middle tibia without large spines on outer surface; hind wings with abundant setae; club two-segmented in both sexes . . . . . (Subgenus *Brachygrammatella*) 2
- Fore wings with bare area immediately beneath marginal vein; middle tibia with large spine or spines on outer surface; female club of single segment; hind wings with setae in two or more distinct lines converging apically . . . . . (Subgenus *Pseudbrachygramma*) 3
2. Marginal vein four times as long as wide; setae on marginal vein rather fine and numerous (about 20), continuing on to wing blade in a triangular cluster beneath marginal vein. . . . . *nebulosa* Girault.

4. Fore wings dusky with contrasting transverse hyaline band across wing blade just distad of venation; body colored yellow and black with silver band on dorsum of abdomen near apex; abdomen long, ovipositor well developed. . . . . *speciosissima* (Girault).
- Fore wings not so patterned. . . . . 5.
5. Fore wings with basal one-third naked; submarginal vein with an abrupt break in middle; marginal vein about two and a half times as long as wide and without thick setae on outer margin. . . . . *indica* sp. n.
- Fore wings with basal one-third setose; submarginal vein of uniform width and contiguous; marginal vein two times as long as wide and with thick setae on outer margin. . . . . *perplexa* (Girault).



FIGS. 1-2

- Marginal vein about three times as long as wide; about eight large, coarse setae on marginal vein, approximately the same number underneath stigmal and apex of marginal vein. . . . . *salutaris* Doult.
3. Ovipositor extended anteriorly in membranous pouch beneath thorax and forward of midcoxal bases. . . . . *ventralis* Doult.
  - Ovipositor not extended forward beneath thorax. . . . . 4

*Brachygrammatella indica* sp. n. (Figs. 1 and 2)

*Female*.—Head yellowish, slightly wider than long in facial view; frontovertex wider than long; ocelli red, arranged in obtuse triangle, basal ocellus separated by about twice its diameter from eye rim and about its diameter from occipital margin; eyes red and smooth; antennae inserted just above the lower level of eyes; space between antennal sockets about one-third the width of frons between eyes; subocular sutures distinct; mandibles with

three acute teeth. Antennae (Fig. 1) yellowish; scape long, about three times as long as wide; pedicel longer than wide, longer than ring and funicle segments combined; basal two ring segments short; funicle 2-segmented; club entire, slightly more than two times as long as wide, apex pointed. Thorax yellowish except sides of metanotum and propodeum, pleural and sternal regions which are dark-brown. Fore wings (Fig. 2) hyaline except an infuscated patch below the marginal vein, slightly less than two times as long as wide, broadly rounded at apex; submarginal vein with an abrupt break in middle; marginal vein about two and a half times as long as wide and with twenty-one setae; basal one-third of fore wing naked; marginal fringe short, spaced by a distance equal to one-half their length. Hind wings hyaline; marginal fringe long, about one-half of wing width. Legs slightly infuscated; tarsi 3-segmented. Abdomen dark except base of dorsum which is yellowish; ovipositor concealed, arising from basal one-third of abdomen.

Female length: 0.8 mm.

Holotype ♀; 2 ♂, 1 ♀ paratypes, INDIA: Uttar Pradesh, Aligarh, University campus, ex eggs of *Oxyrachis tarandus* Fabr. on *Cassia fistula*, 18-9-1974 (M. Younus Khan). Material in Zoological Museum, Aligarh Muslim University, Aligarh, India.

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#### ON THE FIRST GILL ARCH OF *CHANNA* SPP. (FISHES: CHANNIDAE)

THE number of rakers on the first gill arch is of taxonomic value in some fishes<sup>1-4</sup>. In the carnivorous murels, in which they are in the form of dentigerous patches (Srinivasachar<sup>5</sup>), their shape and distribution pattern also are of significance. We for the first time find that the epibranch of the first gill arch reveals diagnostic features.

Srinivasachar<sup>5</sup> first drew attention to the taxonomic importance of the first gill arch in four species of *Channa* (as *Ophicephalus*), viz., *C. marulius*

(Ham. Buch., 1822), *C. striata* (Bloch, 1793), *C. punctata* (Bloch, 1793) and *C. orientalis* Bl. and Schn., 1801 (as *O. gachua* Ham. Buch., 1822). The present note is the outcome of a detailed examination of the first gill arch in a series of specimens of each of the above species.

The first gill arch of either side is connected ventrally to the median ossified copula 2 through cartilage. The gill rakers, in the form of dentigerous patches, are arranged in two parallel rows which, since the arches are directed obliquely backwards from their points of attachment, can for convenience be referred to as the outer and inner rows. The inner row is adjacent to the second branchial arch while the outer row is away from it.

Although Srinivasachar<sup>5</sup> stated that in *C. marulius*, the outer row of dentigerous patches does not extend on to the hypobranch unlike in the three other species, we observe that even in the former species the outer row does extend over the entire length of the hypobranch (Fig. 1 A). In *C. orientalis* these patches extend only to the distal end of hypobranch (Fig. 1 D), in *C. punctata* they extend to about half the length of hypobranch (Fig. 1 C) while in *C. marulius* and *C. striata* they extend down to below half the length of hypobranch (Fig. 1 A, B) in the direction of the ceratohyal of the hyoid cornu.

In *C. marulius* and *C. striata* the outer row of dentigerous patches at the other end extends some distance up along the outer side of epibranch and also backwards along its base (Fig. 1 A', B'). In *C. punctata* they do not extend backwards along the base of epibranch but there will be one (Fig. 1 C') or two patches at the anterior end of its base, while in *C. orientalis* there are 1-3 very small patches restricted to the anterior end; some specimens are devoid of them (Fig. 1 D').

In *C. orientalis* the inner row of dentigerous patches extends only up to the distal end of ceratobranch (Fig. 1 D), while in the three other species, it extends to the base of the epibranch (Fig. 1 A, B, C).

In *C. marulius* and *C. striata* (Fig. 1 A, B), the two rows of dentigerous patches are closer than in the other two species. In *C. striata* they are almost confluent in the middle of ceratobranch. In *C. punctata* and *C. orientalis* the space between the two rows is wider (Fig. 1 C, D), particularly in the latter species.

The shape of the dentigerous patch on the outer side of the base of the epibranch is characteristic in *C. marulius*, *C. striata* and *C. punctata* (Fig. 1 A', B', C'); in *C. orientalis* it is absent (Fig. 1 D').

<sup>2</sup> Swarup<sup>2</sup> has described the epibranch of first gill arch in *C. punctata*. We observe that the shape of the epibranch is characteristic of each species (Fig. 1 A', B', C', D'). In *C. punctata* it bears a process extending back from its lower edge (Fig. 1 C').

The pharyngobranch is fused with the epibranch in *C. marulia*, *C. striata* and *C. punctata*, whereas in *C. orientalis* it is separate (Srinivasachar<sup>3</sup>). Earlier workers have not drawn attention to the unossified distal end of the pharyngobranch in *C. marulia*, *C. striata* and *C. punctata*. The pharyngobranch is completely ossified in *C. orientalis*, in which, on the other hand, there is a cartilage between the epibranch and pharyngobranch.

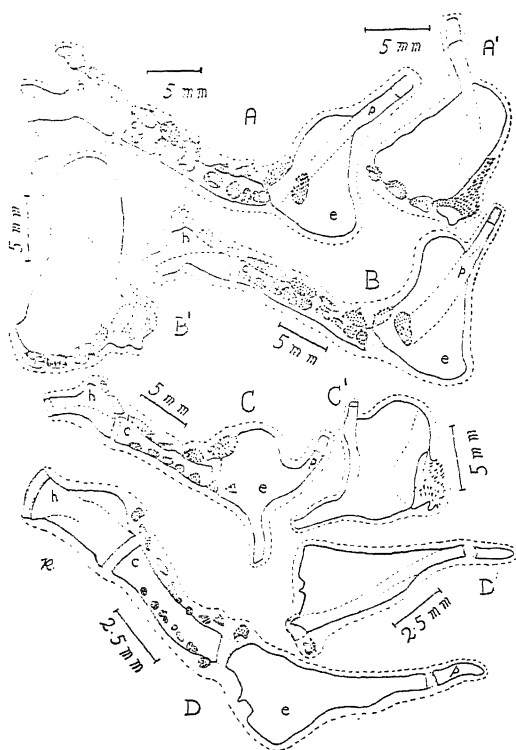


FIG. 1. *Channa* spp., first gill arch of right side and detached epibranch. A, A': *C. marulia* S.L. 249 mm. B, B': *C. striata* S.L. 219 mm. C, C': *C. punctata* S.L. 158.5 mm. D, D': *C. orientalis* S.L. 105.5 mm.

A, B, C and D show hypobranch (*h*), ceratobranch (*c*), epibranch (*e*) and pharyngobranch (*p*) (latter two fused except in *orientalis*—D').

A', B', C' and D' show outer view of epibranch and pharyngobranch. Stippled areas indicate cartilage; broken line around arch represents outline of tissue surrounding it.

The number of dentigerous patches in the four species is presented in Table I.

TABLE I

Number of dentigerous patches on first gill arch (left side)

| Species              | Hypo-branch | Cerato-branch | Epi-branch | Total |
|----------------------|-------------|---------------|------------|-------|
| Outer Row:           |             |               |            |       |
| <i>C. marulia</i>    | 3-5         | 5-7           | 3-7        | 13-17 |
| <i>C. striata</i>    | 3-5         | 4-6           | 4-6        | 12-16 |
| <i>C. punctata</i>   | 1-3         | 3-5           | 1-3        | 6-10  |
| <i>C. orientalis</i> | 1-2         | 4-7           | 0-3        | 6-10  |
| Inner Row:           |             |               |            |       |
| <i>C. marulia</i>    | 0           | 6-8           | 1-2        | 7-9   |
| <i>C. striata</i>    | 0           | 6-9           | 1-2        | 7-10  |
| <i>C. punctata</i>   | 0           | 5-8           | 2-4        | 7-11  |
| <i>C. orientalis</i> | 0           | 5-8           | 0          | 5-8   |

The following key will aid in distinguishing the four species based on a few characters observed on the first gill arch:

- Number of dentigerous patches in outer row more than 11 .. 2  
Number of dentigerous patches in outer row less than 11 .. 3
- Dentigerous patch on outer side of base of the epibranch extends up along edge and is elongate (Fig. 1 A') .. *C. marulia*  
Dentigerous patch on outer side of base of the epibranch does not extend up along edge and is circular (Fig. 1 B') .. *C. striata*
- Epibranch has process extending back from its lower edge (Fig. 1 C, C') .. *C. punctata*  
No process extending from lower edge of epibranch (Fig. 1 D, D') .. *C. orientalis*

We thank Mr. K. Ravindranath, Research Fellow, for assistance in preparing figures. One of us



### A CHEAP SUBSTITUTE FOR GLASS COVERSLIPS IN ROUTINE MICROSCOPIC STUDIES

THE conventional glass coverslips being very fragile are found to be very uneconomical in routine microscopic work. It was, therefore, thought worthwhile to try widely available polythene films or cellophane as a substitute. The results of the studies are presented below :

Polythene films of different gauges (200, 300 and 700) were cut into 18 mm squares, and compared with those of glass coverslips (No. 0 and No. 1). For the studies, various biological materials like fungal spores, pollen grains, nematodes and their eggs and plant chromosomes were observed using polythene and glass coverslips. Cellophane was, however, discarded as it got wrinkled in contact with the mounting media.

It is seen from the photomicrographs (Fig. 1) and their comparative rating (Table I) that the materials under polythene coverslips are as clear as those under glass. Under 700 gauge, however, there was reduction in light transmittance but not clarity.

In addition to the unbreakable nature, the polythene coverslips were found to be unaffected by various reagents used in mounting media or the amount of heating during slide preparation. The adherence of the polythene coverslips to each other can be avoided by cutting these as and when required from a roll of 18 mm wide.

The usefulness of the polythene coverslips in the preparation of permanent slides is under scrutiny. Manufacture of better quality polythene

rolls of uniform thickness will popularise the use of these coverslips in the laboratories.

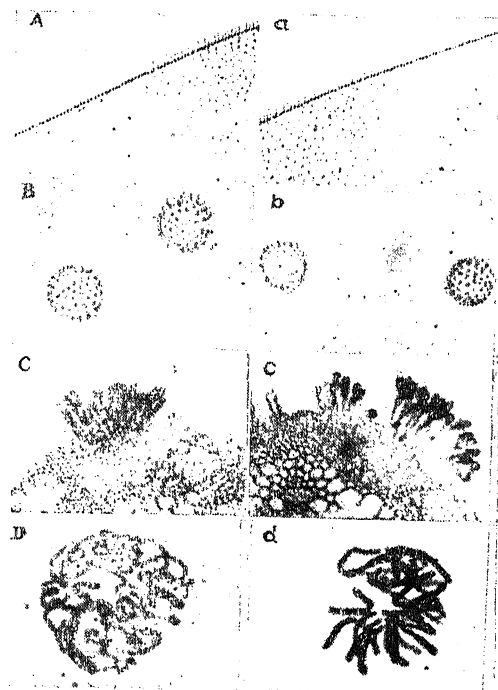


FIG. 1. Photomicrographs taken using glass (A, B, C, D) and polythene (a, b, c, d) coverslips. A, (a): Inner wing margin of *Dacus* sp.,  $\times 100$ ; B, (b): Pollen grains of *Tagetes erecta*,  $\times 400$ ; C, (c): Telium of *Puccinia graminis tritici*,  $\times 400$ ; D, (d): Chromosomes of *Allium cepa*,  $\times 1,000$ .

TABLE I  
Relative efficiency of different coverslips

| Type of coverslips | Refractive Index | Removal of air bubbles | Clarity | Number of pieces (18 $\times$ 18 mm) per 100 g | Cost per 100 g Rs. P. | Price per 100 pieces Rs. P. |
|--------------------|------------------|------------------------|---------|--|-----------------------|-----------------------------|
| <i>Glass</i>       |                  |                        |         |  |                       |                             |
| No. 0              | 1.51             | Difficult              | Good    | 1285   | 85=71                 | 6=67                        |
| No. 1              |                  | Difficult              | Good    | 821  | 85=71                 | 10=44                       |
| <i>Polythene</i>   |                  |                        |         |  |                       |                             |
| 200                | 1.47             | Easy                   | Good    | 115283   | 2=35                  | 0=0020                      |
| 300                |                  | Easy                   | Good    | 84992  | 2=35                  | 0=0028                      |
| 700                |                  | Easy                   | Good    | 34576  | 2=35                  | 0=0068                      |

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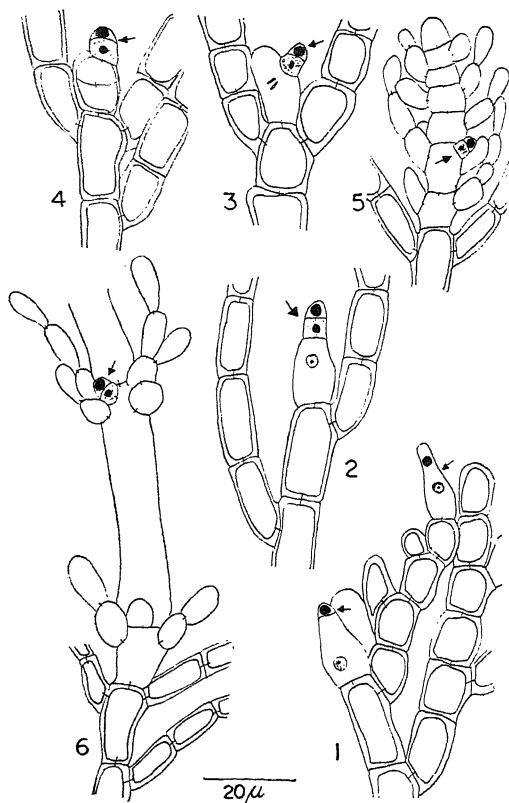
### "ELIMINATION CELLS" IN THE BATRACHOSPERMACEAE

MAGNE<sup>1,2</sup>, while describing the development of the gametophyte in *Lemanea* has reported that meiosis occurs in the apical cell of the upright "Chantransia" filament which produces the gametophyte. After the first division, one of the two resulting nuclei degenerates and is pushed out into a small lateral protuberance which is later cut off as an "elimination cell" ('cellule éliminatrice'). The surviving nucleus then undergoes division and one of the daughters again gets pushed out in a second protuberance which is also cut off as the second elimination cell. Thus, only one haploid nucleus survives and contributes to the origin of the gametophytic phase. In the later paper Magne had indicated that a similar thing could be possible in *Batrachospermum* also, citing Sirodot's<sup>3</sup> figure of *Batrachospermum crouanianum*. Photometric measurement of the DNA content led Hurdelbrink and Schwantes<sup>4</sup> (and Dixon<sup>5</sup>) to suggest that meiosis in an unidentified species of *Batrachospermum* occurred in a position equivalent to that suggested by Magne for *Lemanea*.

This communication reports observations on species of *Batrachospermum* and *Sirodotia* which confirm the suggestions of the authors cited above.

Four species of *Batrachospermum* and one of *Sirodotia* have been studied so far and in all of these, virtually the same pattern of development occurs. A new species of *Batrachospermum* from Mahabaleshwar has been followed in greater detail. In this species apical cells of the "Chantransia" filaments destined to develop into gametophytes, become phialide-like and undergo two successive divisions. At the end of the first division (Fig. 1 right), one of the daughter nuclei degenerates, becomes pycnotic and is finally extruded with a bit of the protoplast as the first elimination cell (Fig. 1 left). Likewise, at the end of the second division also, one of the daughter nuclei degenerates and gets pushed out into a second elimination cell (Fig. 2). The residual portion, containing the

surviving nucleus, enlarges and undergoes division (Fig. 3), resulting in a diad of cells with the two elimination cells perched on top (Fig. 4). Of this diad, the lower cell remains more or less the same in size and produces a sparse whorl of determinate laterals. The upper undergoes a series of divisions and develops into the gametophytic plant (Fig. 5). The products of the upper cell of the diad soon undergo considerable enlargement and develop dense whorls of laterals, characteristic of the gametophytic phase, whose verticillate organization offers a sharp contrast to the monopodial branching of the subtending "Chantransia" filament (Fig. 6). The elimination cells remain clearly distinguishable throughout development because of their characteristic pycnotic nuclei (Figs. 2-6, arrows).

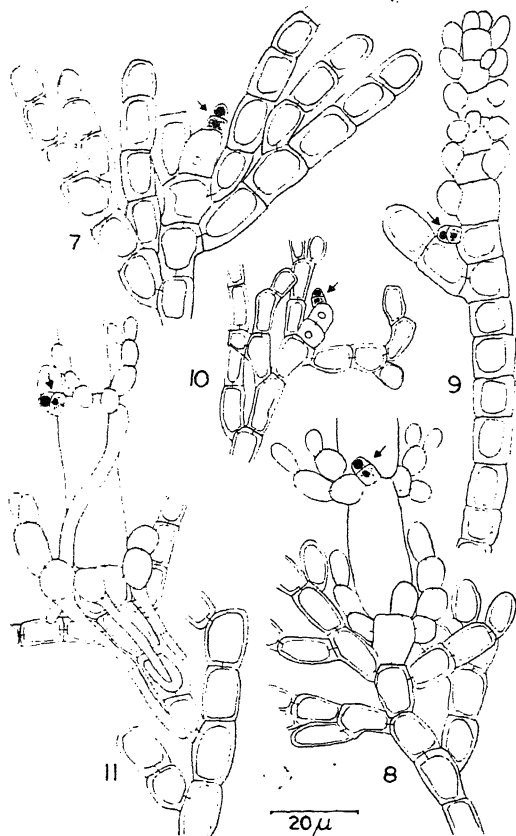


FIGS. 1-6. *Batrachospermum* from Mahabaleshwar. Fig. 1. First division (upper right, arrow) and formation of first elimination cell (lower left, arrow) in apical cells of "Chantransia" filaments. Fig. 2. Formation of second elimination cell (arrow). Figs. 3 and 4. Division and diad formation in the survivor cell. Figs. 5 and 6. Development of gametophyte from the upper cell of diad.

Though all cytological details of the division in the apical cell have not been observed, there is

sufficient indication that this is probably meiotic as suggested by the earlier workers cited. Chromosome counts in the cells of the *Batrachospermum* (haploid  $n=7$ ) and "Chantransia" (diploid  $2n=14$ ) phases provide confirmation of this assumption.

Critical study of three other species of *Batrachospermum*, i.e., *B. ceylanicum* from Jog Falls, Karnatak and *B. sp.* from Matheran, Maharashtra, as well as *B. moniliforme* from Great Britain, has also shown clear-cut elimination cells in all the three (Figs. 7-9, arrows).



Figs. 7-11. Elimination cells in *Batrachospermum* and *Sirodotia*. Fig. 7. *Batrachospermum ceylanicum*, Jog falls. Fig. 8. *B. sp.*, Matheran. Fig. 9. *B. moniliforme*, Great Britain. Figs. 10-11. *Sirodotia huillense*, Mahabaleshwar. (Elimination cells indicated by arrows in all cases.)

*Sirodotia huillense* was collected from Mahabaleshwar. A study of early stages of development in this alga also has shown a similar pattern of development. Here too the elimination cells stand out clearly on account of the pycnotic nuclei (Figs. 10-11, arrows).

Our investigations would thus appear to furnish convincing support to Magne's intuitive assumption that "eliminating cells" could occur in *Batrachospermum*, though it was based only on Sirodot's figure. Four species of *Batrachospermum*, three belonging to section Contorta and one belonging to section Moniliforme (the type section), have elimination cells similar to those of *Lemanea* and the only species of *Sirodotia* we could study also shows this. This clearly indicates that in both the *Batrachospermaceae* and the *Lemaneaceae* the life cycle is of the 'Polysiphonia type', with phases telescoped.

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#### EFFECT OF LIGHT INTERRUPTIONS IN THE LONG DARK PERIODS ON FLORAL INITIATION IN RICE

*Oryza sativa* L., a short-day plant, flowers remarkably earlier when subjected to short-photoinductive cycles (Misra and Khan, 1972 ; Saran, 1945 ; Sircar and Parija, 1949). Light interruptions given in early part of long darkperiod were reported to be inhibitory for floral initiation in dicotyledonous short-day plants like *Xanthium pensylvanicum* and *Perilla ocymoides* (Carr, 1952), *Kalanchoe blossfeldiana* (Bunsow, 1953) and Biloxi soybean (Nanda and Hamner, 1958 ; Wareing, 1954). No work has been done on the effect of light interruptions in long dark period on floral initiation in monocotyledonous short-day plants. The present investigation is aimed at finding out the pattern of floral initiation in (28-day-old) rice plants subjected to 15 consecutive cycles, each comprising of 8 hr photoperiod and 16 hr dark period with interruptions by short intervals of high intensity light as shown in Fig. 1.



Seedlings (21-day-old) of one late-winter rice, BAM 3, were transplanted into earthen pots (25 X 25 cm) at the rate of 3 per pot. The plants were thinned to 4 in each pot. The pots were grouped into five equal groups of 5 pots per group. At the age of 25 days, the optimum age of photoresponsiveness (Misra and Khan, 1973), each group of plants were subjected to 15 consecutive cycles, each comprising of 8 hr daylength and 16 hr darkness. The light interruptions (Fig. 1) were given with fluorescent tubes providing illumination of approximately 500 ft-c at the upper leaf surfaces of the plants. The shoot apices of plants were then examined by microtomy and compared with the controls. The transitional stage, *i.e.*, elongating shoot apex bearing rachillae primordia (Misra and Khan, 1970) was taken as the criterion for floral initiation.

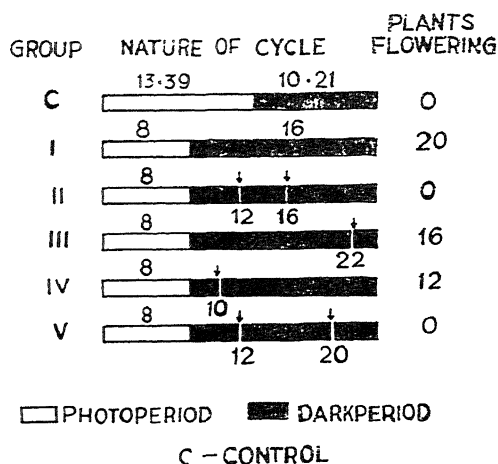


FIG. 1. Cycles comprising of 8 hr photoperiod and 16 hr dark period with 15 minute light interruptions given at different points in the dark period and flowering response to 15 cycles each. Numerals above open areas represent duration of photoperiod, above dark areas duration of dark period. Arrows indicate 15 minute light interruptions.

Plants grown under normal photoperiodic cycles each comprising of 13:39 hr daylength and 10:21 hr nightlength remained vegetative throughout the period of study (Fig. 1, Group C). The plants subjected to 24 hr cycles, each comprising of 8 hr photoperiod and 16 hr dark period with 15 min. light breaks given at 12 hr and 16 hr points after commencement of darkperiod too remained vegetative (Fig. 1, Group II). Plants receiving 15 cycles, each comprising of 8 hr photoperiod alternated with 16 hr uninterrupted dark period, showed floral initiation in all the plants (Fig. 1, Group I), whereas, floral initiation was 60% in plants receiving cycles of 8 hr photoperiod plus 16 hr dark period with

light interruptions at 10 hr point (Fig. 1, Group IV). Floral initiation was completely suppressed in the set of plants receiving light interruptions at 12 hr or 20 hr point after onset of the dark period (Fig. 1, Group V). On the usual assumption that rice plants too have the pigment, 'Phytochrome' in  $P_r$  form which converts to  $P_f$  form when nightlength is conducive for pigment conversion and subsequent synthesis of flowering hormone, the dark period ranging from 2 to 8 hr provided between the two successive light periods in Group II and V appear to be short enough to bring about the pigment conversion and subsequent synthesis of flowering hormone necessary for floral initiation in rice. The control plants receiving 13:39 hr daylength and 10:21 hr dark period remained vegetative evidently for want of a critical nightlength for pigment conversion. Floral initiation in 80% of plants in Group III and 60% of plants in Group IV hints that a dark period of 14 hr duration is, perhaps, not enough to bring about the pigment conversion and subsequent hormone synthesis in rice plants.

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#### ANTHER DEVELOPMENT IN *CLEOME* *TENELLA* LINN.

WHILE studying the embryology of *Cleome tenella* Linn., a small herbaceous plant, occurring in the sandy tracks of Bapatla, Andhra Pradesh, the authors observed some features of interest in the anther tapetum.

The anther is tetrasporangiate. Figure 1 represents an early stage in the development of the anther, dis-

playing in transverse section the 1-celled archesporium in each of its corners. Periclinal divisions in the plate of archesporial cells results in a layer of primary parietal cells towards the outside and a layer of primary sporogenous cells towards the inside (Fig. 2). The cells of the latter undergo a

of anther lobe. Note the absence of fibrous thickenings in endothelial cells,  $\times 400$ .

(*arc*, archesporial cell; *end*, endothecium; *epi*, epidermis; *ft*, fibrous thickenings; *ml*, and *ml*<sub>2</sub>, middle layers; *msmc*, microspore mother cells; *mst*, microspore tetrads; *ppc*, primary parietal cells; *pspc*, primary sporogenous cells; *sg*, starch grains; *spl*<sub>1</sub> and *spl*<sub>2</sub>, secondary parietal layers; *ta*, tapetum.)

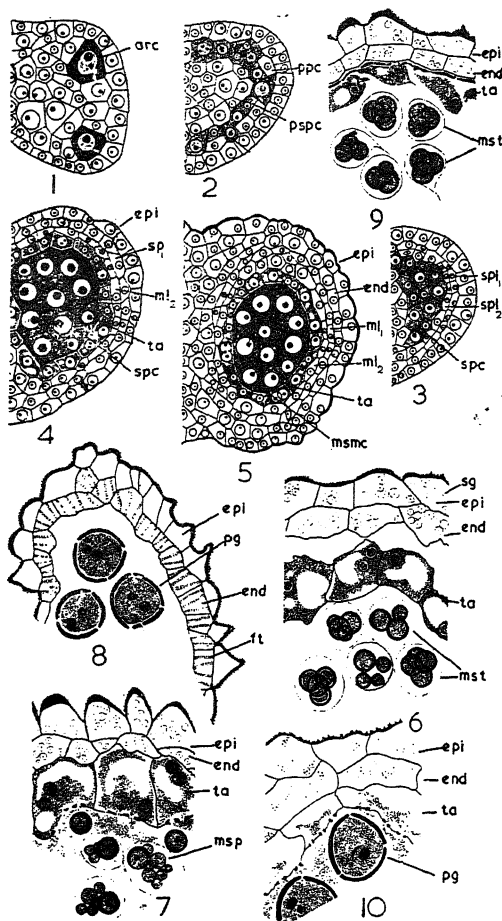
few divisions to produce a mass of sporogenous cells (Figs. 3, 4), while the cells of the primary parietal layer divide both periclinal and anticlinally to produce a 4-layered anther wall (Figs. 3, 4, 5) conforming to the Basic type of development of Davis<sup>1</sup>. Thus at the microspore mother cell stage the wall of the anther shows epidermis, endothecium, two middle layers and secretory tapetum (Fig. 5). The young tapetal cells are, as a rule, 1-nucleate. However, some of them were 2-nucleate even before the onset of meiosis in the microspore mother cells (Fig. 5). In some anthers, the tapetal cells were considerably enlarged, vacuolated with 1 or 2-nuclei (Figs. 6, 7). In such cases, the inner tangential walls of the tapetal cells manifest perforations by the time the microspores are formed (Fig. 7), thereby facilitating the infusion of their viscous cytoplasmic contents into the sporangial cavity. Among the members of Cappariaceae such feature has been recorded for *Capparis grandis*<sup>2</sup>. In normal anthers the tapetum is completely absorbed *in situ* by the time the pollen grains are 2-nucleate (Fig. 8).

The middle wall layers are compressed, soon get crushed and absorbed by the time the microspore tetrads are formed (Fig. 9). The cells of the endothecium enlarge and acquire fibrous thickenings by the time the pollen grains are 2-nucleate (Fig. 8). However, in those anthers, where there is hypertrophy of tapetal cells, the endothelial cells lack fibrous thickenings (Fig. 10). Both in normal and abnormal anthers the outer tangential walls of the epidermal cells, from the time of tetrad organisation, are covered with thick spinescent cuticle (Figs. 6-10). Accumulation of starch grains was observed both in the epidermal and the endothelial cells (Figs. 6, 7, 9, 10).

Meiosis in the microspore mother cells is of the simultaneous type resulting in tetrahedral, isobilateral or decussate tetrads (Fig. 6); the tetrahedral tetrads being more predominant (Fig. 9). Polyads of five to eight microspores of varied sizes occur in such anthers where the tapetal degeneration is abnormal (Fig. 7).

The mature pollen grains are 2-nucleate, tricolpate, with thick smooth exine and thin intine (Figs. 8, 10). About 40% of the pollen grains remain sterile.

The authors are thankful to Prof. A. S. Rao for providing facilities, Dr. B. S. M. Dutt for



FIGS. 1-10. *Cleome tenella* Linn. Fig. 1. T.S. Part of young anther showing 1-celled archesporium,  $\times 300$ . Fig. 2. L.S. anther lobe showing periclinal division of archesporial cells. Note some of the archesporial cells are yet to divide,  $\times 300$ . Figs. 3-5. T.S. anther lobe showing the formation of wall layers and sporogenous cells,  $\times 300$ . Fig. 6. T.S. Part of anther lobe with microspore tetrads, microspores of varying sizes, enlarged tapetal cells and starch grains in the epidermal and endothelial cells  $\times 400$ . Fig. 7. T.S. part of anther lobe with hypertrophied tapetal cells. Fig. 8. T.S. part of anther lobe with fibrous endothecium and 2-nucleate pollen grains,  $\times 400$ . Fig. 9. T.S. part of anther lobe showing microspore tetrads, secretory tapetum, crushed middle layers, endothecium and epidermis. Note the accumulation of starch grains in epidermal and endothelial cells,  $\times 300$ . Fig. 10. T.S. part

discussions. Shri E. Nageswara Rao for providing fixed material and to the authorities of C.S.I.R., New Delhi for the award of Junior Research Fellowship to one of us (B. H. Rao).

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## TWO MORE ADDITIONS TO INDIAN ASCOMYCETES

THE two fungi described in this paper were encountered during the course of an investigation of the Coprophilous microorganisms. Several fungi were isolated and were sent to Commonwealth Mycological Institute, Kew, England, for identification. A scrutiny of available literature showed that *Lophotrichus bartlettii* Malloch and Cain and *Ascodesmis nigricans* Van Tieghem are unrecorded from India. This was also confirmed from the records available at I.A.R.I., New Delhi.

In the present isolate of *Lophotrichus bartlettii* (Massee and Salmon) Malloch and Cain Syn. *L. brevirostratis* L. Ames (Fig. 1) perithecia were

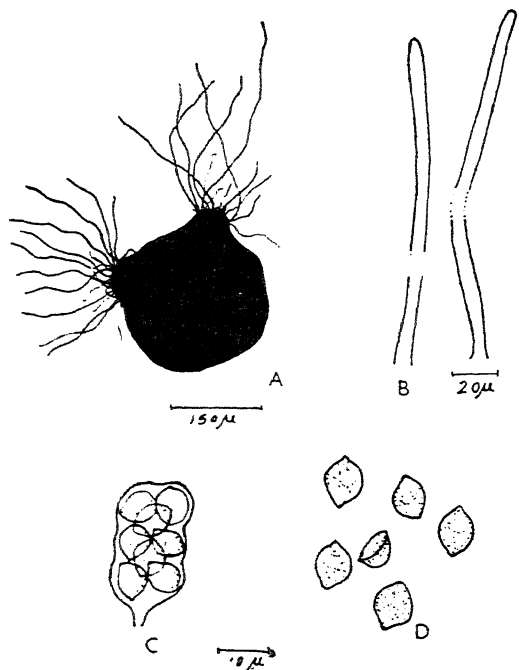


FIG. 1. *Lophotrichus bartlettii*. A. Perithecium; B. Terminal hairs; C. Ascus; D. Ascospores.

smaller (199.5–226 μ to 133–159 μ) in size in comparison to the type species<sup>1</sup>. Ascospores were also broader, i.e., 6.3–7.9 × 6.3 μ in size. This species was reported from the type locality<sup>1</sup>: Edwards County, Kans, on rat dung. This is the first report of this species from the Kangaroo dung collected from the Zoo at Gwalior, M.P. Living culture of this isolate has been deposited at C.M.I., Kew, England, No. IMI 179853.

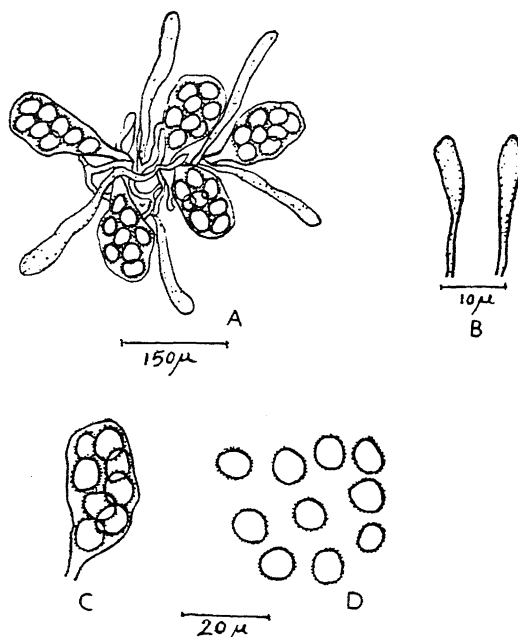


FIG. 2. *Ascodesmis nigricans*. A. Ascocarp; B. Paraphyses; C. Ascus; D. Ascospores.

*Ascodesmis nigricans* is another interesting isolate (Fig. 2), was obtained from Guinea-pig excreta. As this genus is of very rare occurrence and was first reported in 1876 by Van Tiegh. It was kept as a very primitive member of the family Agaricaceae. No literature was available on this genus in our laboratory and was confirmed from C.M.I., Kew, England, under Accession No. IMI 179865.

The authors are grateful to Prof. S. B. Saksena, for encouragement and providing the laboratory facilities. Thanks are also due to Dr. Stockdale, Director, C.M.I., and Dr. Von Arx Centraal bureau Voor Schimmel culture, Baarn, for their kind help in the identification of the isolates.

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### WIDESPREAD OCCURRENCE OF *BEAUVARIA* *BASSIANA* ON RICE PESTS

DURING the survey of microbial diseases of rice pests occurring at Central Rice Research Institute, Cuttack 6, the following were found to be affected by *Beauvaria bassiana*, the white muscardine fungus.

1. *Rice stem borers*: Dead hearts collected from the rice fields when split open revealed the presence of dead larvae of *Tryporyza incertulas* and *Chilo auricilius* which were found overgrown by a white non-sporulating mycelium. The mycelium was seen breaking through intersegments (Fig. 1, a).
2. *Leaf eating caterpillar*: Of the leaf eating caterpillars, *Paranara* sp. was found to be affected by *B. bassiana* (Fig. 1, b). Microscopic examination of the dead larvae showed large number of spores. The spores were single celled borne on a zigzag conidiophores found in clusters.
3. *Green leaf hoppers*: Large number of nymphs and adults of *Nephotettix virescens* were dying in multiplication cages of the Institute during August, 1974. The cadavers were covered by a white powdery mycelium consisting of a large number of spores of *B. bassiana* (Fig. 1, c).

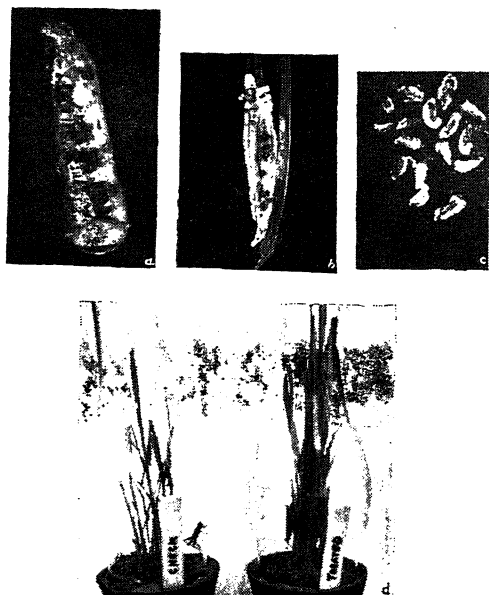


FIG. 1. *Beauvaria bassiana* infected (a) larva of *Chilo auricilius*, (b) larva of *Paranara* sp., (c) green leaf-hoppers and (d) less feeding in *B. bassiana* treated grasshoppers.

The epizootic in the cages was favoured by high humidity due to rains and high temperatures prevailing during the first week of August. However, in the later part of the month when weather

was dry, the hopper mortality declined. In general, the affected host was found to be sluggish and did not feed much.

In all the hosts, the mycelium emerged through intersegments and then covered the whole body. The affected larval and leaf-hopper bodies were hard and brittle and crumbled when pressed. Spore formation was dependent on humidity.

*Isolation and pathogenicity tests.*—Diseased specimens were surface sterilized with 0.1%  $\text{HgCl}_2$  in 75% alcohol for 1–2 min washed in sterile water and plated on Sabouraud's agar<sup>1</sup>. The plates were incubated at 28–30° C. The fungal colonies appeared within 4–5 days, which were small and slow growing. The mycelium was white, fluffy turning to mealy and chalky growth as the colony became old. Spore formation was observed after 2–4 days. Spore measurements of different isolates indicated that they all belonged to one and same species. The culture was identified as *B. bassiana* by Commonwealth Mycological Institute, Kew, United Kingdom.

*Pathogenicity tests.*—The fungal isolates from different species were multiplied on Sabouraud's agar for one week, and then 7–10 days old larvae of stem borers, *Paranara* sp. and adults of green leaf-hoppers were allowed to come into contact with the respective colonies for 10–15 min to cover them with spore mass. The treated stem borer larvae were transferred to rice cut-stem pieces for feeding. *Paranara* larvae and the green leaf-hoppers were released separately in caged plants. Daily observations were made for disease development. In 4–5 days nearly 50% of treated stem borer and *Paranara* larvae died, whereas total mortality of leaf-hoppers was obtained in 3 days. The fungus was re-isolated from the affected insects. The three isolates were cross inoculated and found pathogenic to all the three hosts.

*On other rice pests.*—To check whether the fungus was pathogenic to other rice pests, the following species (1) *Sesamia inferens*, (2) *Cnaphalocrocis medinalis* (leaf folder) and (3) *Hieroglyphus* sp. and *Oxya* sp. (grasshoppers) were inoculated with *Tryporyza* isolate as described earlier. All the pests were found susceptible to *B. bassiana* culture and in the case of grasshoppers, feeding declined 24 hr after treatment (Fig. 1, d).

*Beauvaria* spp. are well-known insect parasitic fungi and considerable work has been done to utilise them for biological control of insect pests<sup>1-4</sup>. However, so far no report has been made on the widespread occurrence of *B. bassiana* on rice pests particularly on stem borers. Since rice microclimate favours the growth of this pathogen, experiments

are in progress to evaluate its field efficacy against total rice pests occurring at C.R.R.I. this season.

Thanks are due to U.S.D.A. for financing the Project No. FG-In-468 'Biological Control of Stem Borers of Rice in India' under which the work was carried out and also to Dr. Brady of C.M.I. for identifying the fungus.

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#### HITHERTO UNRECORDED POST-HARVEST DISEASE OF APPLE

DURING the course of study of post-harvest diseases of fruits and vegetables of Warangal (A.P.), the authors found a considerable percentage of apple spoilage due to a brown rot. The spots which were originally small discoloured areas, gradually increased in size, the colour also deepened and finally turned brown (Fig. 1). Isolations from such diseased portions repeatedly yielded the culture of *Torula* stage of *Hendersonula toruloides* Nattrass.

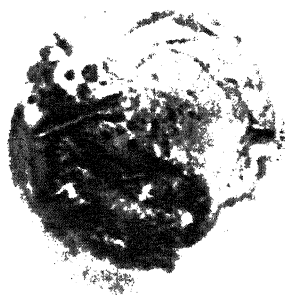


FIG. 1. Photograph of *Pyrus malus* fruit showing symptoms caused by infection of *Hendersonula toruloides* Natt.

*Morphology of the pathogen.*—Colony on P.D.A. spreading, with profuse aerial mycelium white in the beginning; as it ages it attains smoky black colour. Hyphae septate, branched, greyish black, 1.25–3.0  $\mu$  (average 1.5  $\mu$ ) wide; hyphae produce

chains of conidia (thallospores or arthrospores), spores globose to wedge-shaped; oval 4.0–6.0  $\mu$  (average 5.75  $\mu$ ) in diameter, elliptical 8.5–11.75  $\mu$  (average 10.25  $\mu$   $\times$  4.75  $\mu$ ), pycnidia were not observed either on the fruits or in culture.

The pathogenicity of the fungus was established by Granger and Horne (1924) method. When the spore suspension was sprayed on injured and uninjured fruits, the symptoms appeared early in the former one. The fungus was labelled as pathogen only after satisfying Koch's postulates. Perusal of the literature<sup>1-7</sup> revealed that the pathogen has not been reported earlier from India or elsewhere and this is the first report of its occurrence.

Thanks are due to Prof. U. B. S. Swamy for encouragement and providing laboratory facilities.

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#### RAY AND FUSIFORM INITIALS IN BAUHINIA SPECIES

BAILEY<sup>1</sup> noted that fusiform initials constitute about 87.5% of the total area of cambial zone in 60 years old trees of *Pinus strobus*. Wilson<sup>2</sup> calculated the surface area of the wall of fusiform and ray initials in *Abies concolor* and found that the fusiform cells occupy more than 90% by volume in the cambial zone, and later gave a model for the cambium of conifers based on his observations on *Abies*. Similar composition for the cambium was reported by Kozłowski<sup>3</sup>. Butterfield<sup>4</sup> also recorded 95% of fusiform cells in the cambial zone of *Aeschynomene hispida*. Contrary to the above, Ghouse and Yunus<sup>3,4</sup> and Ghouse *et al.*<sup>5</sup> observed that fusiform initials in the cambial zone do not exceed 82% in some arid zone plants, 60% in adult *Dalbergia* and 25% in *Dillenia* species.

Cambial samples collected in August 1973 from six *Bauhinia* species, sectioned in tangential plane and stained in tannic acid-ferric chloride, showed

essentially fusiform cells and ray initials. The arrangement of initials makes the cambium non-storied<sup>1</sup> as in the majority of dicotyledons.

The ratio of the different initials calculated on the basis of the area occupied by them in T.L.S. showed that fusiform cells form about 75% in *B. parviflora*, 71% in *B. malabarica*, 64% in *B. purpurea* and *B. variegata*, 62% in *B. alba* and 54% in *B. triandra* (Fig. 1).

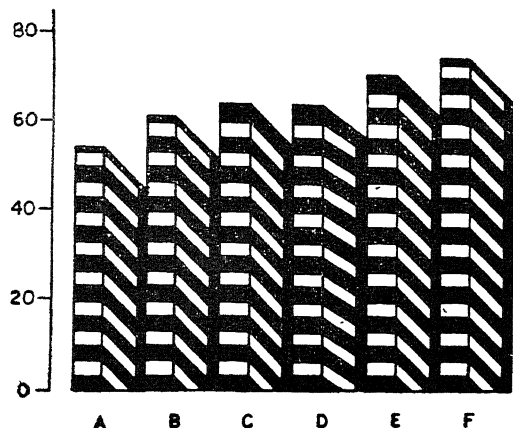


FIG. 1 Histograms showing the percentage area occupied by fusiform initials in the cambial zone of *Bauhinia* species. A, *B. triandra*; B, *B. alba*; C, *B. purpurea*; D, *B. variegata*; E, *B. malabarica*; F, *B. parviflora*.

As in the other cases, studied in this laboratory<sup>3-5</sup>, fusiform cells in Indian tropical trees do not constitute as high a proportion (90% or more), as was reported for conifers<sup>1,6-8</sup> and in *Aeschynomene hispida*<sup>2</sup>.

Authors (M. Y. and M. I.) express their deep gratitude to C.S.I.R., New Delhi, for the award of Senior and Junior Fellowships respectively. We feel grateful to Prof. R. Khan, for providing necessary laboratory facilities.

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## PATHOLOGICAL AND HISTOLOGICAL NOTE ON MANGO MALFORMATION IN EGYPT

MANGO malformation is one of the major diseases in Egypt. It was noticed during 1934, and became severe by 1958. Some Entomologists believe that the causal organism is a mite<sup>5</sup>, others attributed it to the virus<sup>3</sup>. On the other hand, Indian pathologists<sup>6-8</sup> reported that the disease is caused by *Fusarium moniliforme*, and they successfully reproduced the disease with this fungus.

In the present investigation, *Fusarium moniliforme*, *Fusarium* spp., *Botryodiplodia theobromae*, *Nigrospora* sp., *Helminthosporium* sp., *Curvularia* sp., *Alternaria* sp., *Verticillium* sp., *Botrytis* sp., *Cladosporium* sp., *Georrichum* sp., *Pestalotia* sp., *Stemphylium* sp., *Epicoecum* sp., *Monocillium* sp., *Sporobolomyces* sp., and *Merothecium* sp. were isolated from undifferentiated buds, vegetative, and floral malformations.

Certain isolates of *F. moniliforme* were found to be pathogenic and produced vegetative malformation similar to that seen in Upper Egypt, denoting that this fungus is the cause of the disease. However, inflorescence malformation could not be reproduced, without wounds for the entrance of the fungus into the host tissues. These results are in agreement with those reported by Summanwar<sup>7</sup>. On the other hand, Burns<sup>1</sup> and Prasad<sup>4</sup> failed to isolate any fungus from the diseased plants.

Cross and longitudinal sections from malformed tissues vegetative and inflorescence, prepared according to Johansen<sup>2</sup>, revealed the presence of the fungus in the infected tissues, particularly in the cortex layer. However, discoloration of the xylem tissue was developed. Hyphae of the fungus were observed intra- and intercellular spaces and the fungus has the ability to penetrate the host cell mechanically. In the healthy tissues, the sclerotic cells, in the cortex layer of the stem, stain red with safranin, but in the diseased stems, this reaction disappeared. The invasion of hyphae to the pith cells, took place after the cells became less healthy. Hyphae might be seen crossing the cells or lining their walls. The fungus formed globose structures, similar to chlamydospores, particularly in the cortex, after artificial inoculation with the spore suspension. Naturally infected tissues showed

that cell abnormality and formation of globose structures were clear within the cells, particularly in the pith cells. These results however, are not in agreement with Prasad *et al.* who failed to see any fungal growth in the infected tissues.

Growth of *F. moniliforme* *in vitro*, was completely inhibited by Benlate at a concentration of 50 g/l. Topsen, Ortho-Dipholan, Mil-col, Captan, Acri-dione, Ferrated and Vita-vax were next in their inhibition, and Mil-carp and Dithane Z-7, were the least effective fungicides. Chemical control with Captan, Dithane (3 g/l), and Benlate (1 g/l), did not give any disease reduction when sprayed on established trees. In most of the treatments, the malformation was higher than in the control. Summanwar<sup>7</sup> however, stated that he obtained good control of the disease with Captan.

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Agric. Research Centre, ZEINEB M. EL-TOBBY.  
Min. of Agriculture, M. A. ABDEL SATTAR.  
Cairo, Egypt, October 2, 1974.

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#### BOTTOM HEAT—A NEW TECHNIQUE FOR ROOTING HARDWOOD CUTTINGS OF TROPICAL FRUITS

FOR the maintenance of uniformity in the genetic constitution of the selected plants, clonal propagation methods such as cuttings are employed to perpetuate the fruit crops. The propagation of mango and guava through cuttings is besieged with several limitations as the cuttings are difficult to root. Ringing, application of growth regulators to

the shoots before detachment and planting the cuttings under mist appear to give rooting<sup>1-4</sup>. The bottom heat has been successfully used for rooting of hardwood cuttings of temperate fruits<sup>5</sup>. This procedure is tested with mango and guava. For providing the bottom heat to the cuttings, a simple device was constructed with two cylinders of galvanised iron sheet, the outer one being 70 cm in height and 46 cm in diameter and an inner jacket having a height of 68 cm and a diameter of 44 cm. The space between inner and outer containers was packed with glasswool for heat insulation. Another container, which exactly fitted into the inner jacket, also constructed from the same material measuring 35 cm in height, having two electric bulbs at the bottom meant to heat the bottom of the medium. This container was used for planting the cuttings of mango and guava. The temperature in the rooting zone was regulated to be around  $30 \pm 2^\circ \text{C}$  throughout the experiments.

Mango cuttings were collected from the Dashehari seedlings and guava from Allahabad Safeda stool plot. The leaves were removed in the case of mango and the cuttings were trimmed to about 40 cm length by cutting the terminal portion just above a bud. The cut ends and leaf scars were smeared with lanolin paste to prevent desiccation. Guava cuttings were similarly prepared except that two pairs of half leaves were retained. These cuttings were given quick dip treatment with IBA 5000 ppm prepared in 50% ethyl alcohol for 15 seconds and planted in the bottom heat device in bundles of 10 cuttings each. The planting medium consisted of equal parts by volume of finely chopped sphagnum moss and grit, sand (1:1). The cuttings were planted in the medium to a depth of 15 cm. High humidity was maintained around the cuttings by covering them with a polythene tent.

By using this technique, 97% of the mango cuttings rooted in a month's time. The average number of roots per rooted cutting was 16 and they were fibrous. In the control only 15% of the cuttings rooted having one root each on average. With guava hardwood cuttings, 87% success was obtained having 21 roots per rooted cutting, whereas in the control 17% cuttings rooted and had 5 roots per rooted cutting. Thus the rooting of hardwood cuttings of mango and guava by the use of bottom heat treatment during the winter is spectacular. This appears to be the first instance to employ bottom heat for rooting the cuttings of tropical fruit crops like mango and guava. Another point of interest is that by this technique about 400 cuttings can be rooted successfully in an area of 44 cm<sup>2</sup> which is highly economical.

Our thanks are due to Dr. R. N. Singh, Head of the Division of Horticulture and Fruit Technology, for providing the facilities.

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Fruit Technology,  
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## SHORT SCIENTIFIC NOTES

### New Host Records of the Root-Knot Nematode, *Meloidogyne incognita*

The present communication gives the list of certain new hosts of the root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, which have not been reported earlier<sup>1-3</sup>. The specific identification was done by the close examination of the perineal patterns of mature females, taken out from the galled tissues of the roots. Hot acid fuchsin (0.01%) and lactophenol were used as staining and mounting materials respectively. The root infection based on :  
+ = light, ++ = moderate, +++ = heavy, ++++ = severe and size of root-galls ranging from small (S), medium (M) to large (L) were also noted.

*New Host Records*: *Cassia tora* L. (+, S); *Cucumis melo* var. *agrestis* Naud. (++++, L); *Cyperus rotundus* L. (+, S); *Digitaria cruciata* (Nees.) A. Camus, (++, SM); *Gomphrena globosa* L. (+, S); *Mukia maderaspatana* Roem. (++++, L).

*New Reports from India*: *Coronopus didymus* (L.) Sm. (+, S); *Vitis vinifera* L. (++, SM).

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### Chemical Races in Variyali Sowa

Three distinct varieties of India dill fruits, Ghoda (mericarp) Vizag (cremocarp and mericarp) and Variyali sowa (cremocarp) were reported by Shah *et al.*<sup>1,2</sup>. Out of these varieties Vizag sowa fruits were completely free from dillapiolene but was still identified as different from *Anethum graveolens* on the basis of their flavanoid pattern<sup>3</sup>. The Variyali sowa fruits reported were strongly convex cremocarps with narrow wings and dark brown in colour. This variety on cultivation flowered one month earlier than the other two sowa varieties. Gas chromatographic analysis of the oil from this variety showed dihydrocarvone—43%, carvone—21%, limonene—20% and dillapiolene—13%. Thus the presence of double the amount of dihydrocarvone than carvone was reported for the first time.

Another variety of Variyali sowa was received from the market of south Gujarat which is paler in colour and with broader wings. It yielded higher percentage of oil and on cultivation did not show early flowering like the dark variety. This is reported here as pale Variyali sowa containing higher percentage of carvone than dihydrocarvone.

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TABLE I

| Characters                                      | Dark Variyali sowa   | Pale Variyali sowa  |
|---|--|---|
| Colour  | Dark brown with yellowish narrow wings                                   | Pale brown with yellowish broader wings   |
| Shape   | Ovate oblong, dorsally strongly convex cremocarp with or without pedicel | Ovoid lanceolate slightly dorsally compressed cremocarp with or without pedicel |
| Breadth of wings                                | 0.1 to 0.2 mm  | 0.2 to 0.4 mm   |
| Wt. of 100 fruits                               | 600 to 700 mg  | 650 to 730 mg   |
| Percentage of oil obtained on 5 hr distillation | 2.07   | 4.50  |
| Refractive index at 25 °C                       | 1.485  | 1.490   |
| Sp. gravity at 25 °C                            | 0.9440   | 0.9271  |
| Optical rotation [ $\alpha$ ] <sub>D</sub>      | 26.26  | 69.30   |
| Limonene % v/v                                  | 24   | 39  |
| Dihydrocarvone % v/v                            | 35   | 5   |
| Carvone % v/v                                   | 23   | 42  |
| Dillapiol % v/v                                 | 18   | 14  |

Thus these two varieties of Variyali sowa can easily be distinguished with carvone : dihydrocarvone ratio, which is less than 1 in dark variety, while more than 1 in the pale variety. Important morphological characters of these two varieties and composition of the oils obtained by 5 hr distillation is summarised in Table I. No microscopic difference was noted.

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#### A New Host Record for *Curvularia lunata* (Wakker) Boedijn

While surveying the pathogenic fungi of Distt. Jaunpur (U.P.) a leaf-spot disease of *Ipomoea fistulosa* was observed by the authors in August 1974. The leaf-spots are minute, oval, light brown, 1-2.5 mm in diameter and distributed throughout the lamina.

Microscopic examination of diseased leaves revealed the presence of *Curvularia*. The fungus was isolated on sterile PDA and identified as

*Curvularia lunata* (Wakker) Boedijn. The colony colour was dark black and the growth was luxuriant on both PDA and leaf extract agar media.

Pathogenicity tests were conducted on plants of *Ipomoea fistulosa* grown in Botanical Garden of St. Andrew's College. Four to seven days old spore-cum-mycelial suspension of the fungus was atomised on healthy leaves of the host. Symptoms developed after 6 days of inoculation. On re-isolation from the artificially infected leaf-spots the same fungus was obtained.

In the literature so far available, there is no previous record of *Curvularia lunata* on *Ipomoea fistulosa*. Therefore the present note records *Ipomoea fistulosa* as a new host for *Curvularia lunata*.

The authors are thankful to Dr. G. P. Agrawal, Professor of Botany, Jabalpur University and to Dr. Y. B. Singh, Principal, St. Andrew's College, for encouragement. Thanks are also due to Sri Y. N. Srivastava, Sri. F. Abbasi, Dr. A. B. Sinha of St. Andrew's College and to Dr. Kamal of Gorakhpur University, for their kind help.

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March 4, 1975.

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*Fusarium equiseti* (Ida) Sacc., a Fungus Infecting the Okra Petiole Maggot (*Melangromyza hibisci* Spencer)

During the course of studies of a new okra pest, *Melangromyza hibisci* Spencer, natural infection by a fungus attacking the pupal and adult stages of this insect was observed at the Experimental Station of the Indian Institute of Horticultural Research, Hesaraghatta. The dead adults were found adhering to the leaves, whereas the dead pupae were found within the petioles. White mycelial growth of the fungus was observed on these dead pupae and adults.

A profusely sporulating fungus was readily isolated from infected pupae and adults on potato dextrose agar medium and has been identified as *Fusarium equiseti* (Ida) Sacc.

In order to confirm the pathogenicity of this fungus, healthy pupae collected from the field were placed in sterile petriplates and were inoculated with spore suspensions of the fungus. Similarly another set of pupae inoculated only with sterile distilled water served as control. All the pupae inoculated with spore suspension died as indicated by the non-emergence of the adults, while in control, the pupae remained alive and the adults emerged out of them.

This is the first record of a species of *Fusarium* on this okra pest.

The authors are thankful to Dr. G. S. Randhawa, Director, for his interest in this work and to Dr. J. A. Von Arx, Director, Institute for Central Type Culture Collection, Netherlands, for kindly identifying this fungus.

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#### Bacterial Leaf Spot of *Datura metel* Caused by *Xanthomonas campestris* f. sp. *daturi* f. spec. nov.

A severe bacterial leaf spot disease of datura (*Datura metel* L.) was observed near Jaipur in October, 1973. Initially small, round water soaked spots are formed which later turn brown measuring upto 4 mm in diameter. These circular spots are distinctly raised and surrounded by a halo-like zone of yellow colour. The disease is mostly confined to leaves but sometimes dark brown lesions are also formed on petiole and tender stem.

A yellow pigmented fast growing bacterium was isolated from diseased leaves on nutrient agar. Bacterial suspension was prepared in distilled water from 48 hr culture grown on nutrient agar. Two months datura plants were spray inoculated by (i) injury with fine carborundum powder and (ii) without any injury. Typical water soaked spots on injured leaves were observed after 5 days, whereas

on uninjured leaves symptoms appeared after 9 days. Younger leaves were more susceptible than older leaves. Complete symptom expression on both the surfaces of leaves was observed after 15-20 days. Rolling and dropping of leaves started after a month. The bacterium was reisolated and was compared with original one in all respects. This bacterium failed to infect tomato and chilli seedlings in repeated trials but resembled in all morphological and physiological characters to *Xanthomonas vesicatoria* (Doidge) Dowson isolated from chillies. Mathew (1972) also isolated a *Xanthomonas* sp. from datura and reported the similar results. He classified this bacterium as a strain of *X. vesicatoria*.

The culture has been deposited at Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, England (IMI, B 5840) and identified by Dr. J. F. Bradbury as a forma specialis of *Xanthomonas campestris* (Pammel) Dowson. The present bacterium has been named *X. campestris* f. sp. *daturi* f. spec. nov.

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#### A New Host of Powdery Mildew

A new powdery mildew disease was recorded on *Phaseolus trilobus* Ait. (Leguminosae; sub-family: Papilionaceae) at the College of Agriculture Farm, J.N. Krishi Vishwa Vidyalaya, Jabalpur, M.P., in August, 1973: The mildew usually appears epiphytically but sometimes it occurs amphigenously. Pedicel and pod are also affected. As the season advances, necrosis develops in the affected parts and the affected leaves defoliate.

*Morphology of the fungus*: Mycelium septate, hyaline, branched and superficial; conidiophores simple, erect, slightly swollen at the tips and bear long chains of conidia; conidia oval to oblong, hyaline and measure  $19-35 \times 10-20 \mu$ . Cleistothecia absent.

The fungus has been identified as *Acrosporium* sp. *Phaseolus trilobus* is a new host of *Acrosporium* sp. The specimen has been deposited in the herbarium of the Department of Plant Pathology, J.N. Krishi Vishwa Vidyalaya, Jabalpur, M.P.  
Department of Plant Pathology, S. C. VYAS.  
J.N. Krishi Vishwa Vidyalaya, L. K. JOSHI.  
Jabalpur 482 002, M.P., February 28, 1975.

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## REVIEWS AND NOTICES OF BOOKS

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**Essentials of Computer Programming in FORTRAN IV.** By N. N. Biswas. (Radiant Books, 511, Upper Plaaee Orchard, Bangalore 560 006), 1975. Pp. xi + 125. Price Rs. 16-00.

In a world flooded with books on Fortran programming, one may well be tempted to ask 'why one more?' Biswas's book *Essentials of Computer Programming in FORTRAN IV* adds to the already extensive literature available on the subject. Yet, for many reasons, 'Essentials' is a useful addition.

The author state in his preface that this is 'a simple text book for the beginner' and this steady focus on the beginner's needs is precisely the book's major virtue. It gives the beginner the kind of instruction he requires to be able to start writing workable programs without getting him involved with too much theory. At the same time, the requisite theoretical underpinning is provided in the book's lucid explication of the subtler aspects of programming.

The book is divided into nine chapters, with three appendices. Chapters 1 through 8 describe number representations, arithmetic operations and expressions, relational operators and logical expressions, input output statements, arrays, control and decision statements, the DO loop and subroutines. The concluding chapter, *Illustrative programs*, consists of four well-chosen examples, each of which is preceded by a detailed description of the problem selected. The programs have been developed to bring out clearly many of the salient features of Fortran. Mention must also be made here of the interesting problems set at the end of each chapter (except Chapter 8 on subprograms) for the learner to work out on his own. The three appendices provide a ready and easy guide to the beginner as well as information on what is available on system 360 44 PS. Appendix A on flow charts, without giving undue importance—to its subject, describes briefly but clearly the structure of flow charts in the context of their limited usefulness for the purpose of documentation of a program. Appendix B—*Mathematical function subprograms*—is a useful list of functions of programs available on 44 PS, and Appendix C gives a set of the typical forms of different kinds of statements—again a very useful part of the book.

Throughout the book, the employment of a kind of inductive method generalisation following specific illustrative examples serves to carry the learner along with the author and helps to fix ideas in the learner's

mind. This is an excellent method of representation from the pedagogical point of view, and another of the book's plus points.

Now for a brief consideration of its minus points. At a few places in the book, organisation breaks down and the author is forced to refer to items which are introduced later in the book. (For example, the H format has been used on page 41 but formal definition and explanation appears only on page 43). Occasionally, explanations and illustrations are inadequate, e.g., in discussing Arithmetic operators, understanding would have been enriched if the hierarchy of operations had been illustrated with more examples. Similarly, the example for the manipulation of vector on page 107, though good in the sense that it is illustrative of several aspects of programming, fails to give a detailed break-up of instructions and is impoverished to that extent. As a book for self-instruction, 'Essentials' is ideal except for the omission of a key to the problems, something the learner would appreciate a great deal.

On the whole, it is a pleasure to read a book, born out of a practical experience in programming, written in a simple, readable style; it contains few typographical errors. Certainly a 'must' book for the beginner who needs to master the art of writing successful program.

N. RAMANI.

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**Graphic Representations of the Periodic System During One Hundred Years.** By Edward G. Mazurs. (University of Alabama Press, Alabama 35486). Pp. xii + 251.

The importance of the periodic table in the understanding and teaching of the properties of the chemical elements and their compounds is enormous and it is no wonder that the human mind has exercised itself considerably in developing the most convenient way of arranging the elements. As the author points out there have been nearly seven hundred periodic tables published during the past one hundred years. The author has made an excellent attempt in summarising the entire literature concerning the periodic tables from the days of Louis Bernard Guyton de Morveace (1782) and Dobereiner (1829) to the present day. The two most significant points in the history of the development of the classification of elements have been the publication of the periodic table of Mendeleev based on the atomic weights of elements (later

modified making the atomic number as the basis) and the tables based on the electronic configuration of atoms. These developments have been critically discussed by the author. The book with a number of periodic charts neatly reproduced and containing a large number of literature references would certainly be a good addition to any library.

G. K. NARAYANA REDDY.

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**Advanced Soil Physics.** Edited by Dr. C. Dakshinamurthy. (I.C.A.R. Publication, New Delhi), 1972. Pp. x + 249. Price Rs. 15-00.

This book contains the various aspects of Soil Physics such as particle size distribution, colloidal properties of clays, soil structure, flow of water through soils, etc. The editor deserves full compliments for having presented different topics in lucid and illustrative way. The theoretical treatment of the subject is good. Apart from topics in Soil Physics, certain aspects of Agricultural Meteorology such as the effect of physical environment on plant growth, boundary layer phenomena in the earth atmosphere system and the thermal balance of the earth surface are also clearly explained.

This book may be very useful for Agricultural Scientist as it includes some basic concepts of Mathematics and Physics required to understand the topics dealt in Soil Physics. This book may also be conveniently revised into an advanced textbook by incorporating some of the recent advances made in the subject so that it will be more useful to the student of Soil Science and allied subjects.

R. V. RAMAMOCHAN.

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**Water Resources of India and Their Utilisation in Agriculture.** By C. Dakshinamurthy, A. M. Michael and Shri Mohan. (Water Technology Centre, Indian Agricultural Research Institute, New Delhi), 1973. Pp. 400. Price Rs. 25-00.

Water is a precious resource to be used with utmost care. However this realisation has come about only in recent times and therefore attempts are being made all over the world to assess water potential and to conserve and properly utilise water. In this context the present publication is a welcome addition to the all too scarce literature on the subject in India. The book brings together the available information on water resources in India and discusses the need and ways of their optimal utilisation. The water resources are classi-

fied into surface water resources and ground water resources. The former chapter discusses the past, present and future of the utilisation of the water of the river systems and the latter chapter reviews the ground water resources and their proper exploitation. The rest of the chapters discuss the techniques of soil management and crop management for efficient water use, moisture conservation in dry farming areas, the construction and maintenance of irrigation wells and the conveyance and application of water on the farm. A very useful chapter on the reclamation of water logged and salt affected areas follows. The last chapter discusses the economics of water resource utilisation. A list of useful references is given. The book has brought together much useful information and statistics scattered in the literature and would therefore be very useful to all those concerned in the exploitation, utilisation and conservation of water.

K. RAMAKRISHNAN.

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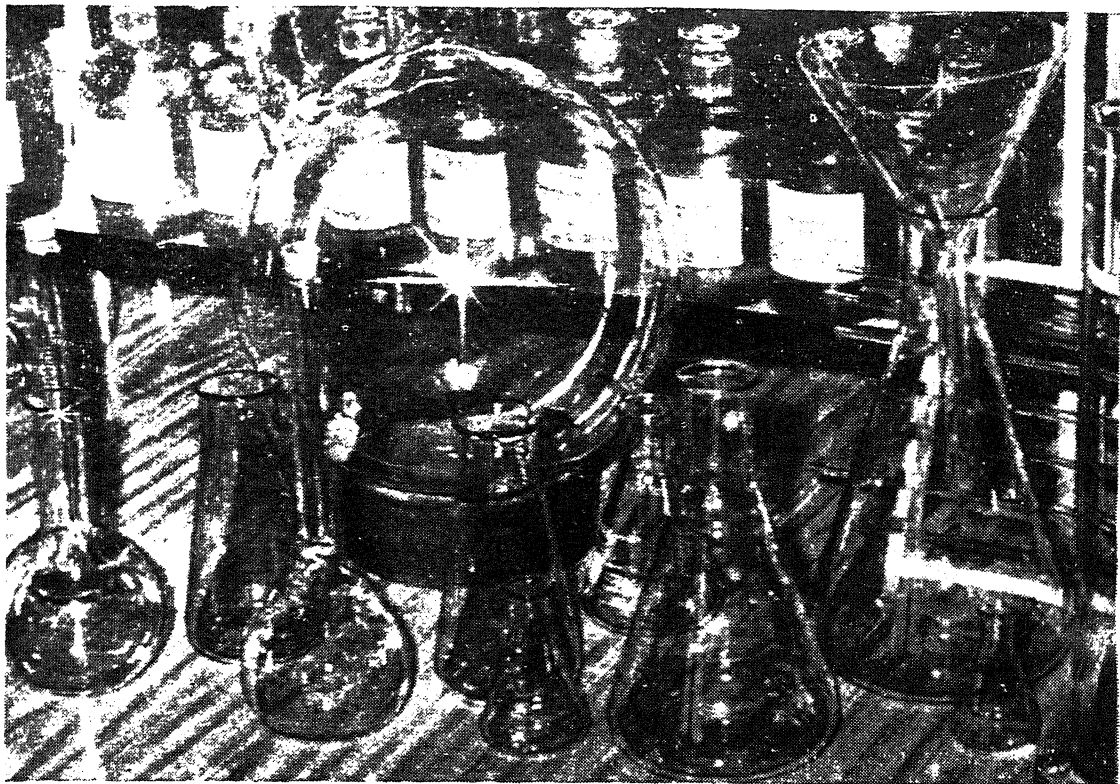
**Design and Evaluation of Irrigation Methods.**

By A. M. Michael, Shri Mohan and K. R. Swaminathan. (Water Technology Centre, Indian Agricultural Research Institute, New Delhi), 1973. Pp. 208. Price Rs. 15-00.

India has the largest irrigation network in the world and it is expected that by further exploitation of our water resources, the area under irrigation could be raised to 50% of the sown area by the end of this century. There is however much need for sophistication in the utilisation of irrigation water. A great deal of research has been done on the qualitative aspects of water use all over the world including India. The present book is therefore very timely as it brings together much useful information on various methods of irrigation and critically evaluates them. After an introductory chapter on the basic variables and efficiencies in irrigation methods, the later chapters deal with border irrigation, furrow irrigation, sprinkler irrigation and drip irrigation. A list of useful references is provided. Four appendices provide useful data on water measurement, computer programming, frictional loss in irrigation pipes and specification of sprinkler equipment.

The book would be found useful by all those concerned with irrigation and water use.

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# GEOBIOS

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## ABSTRACT

Infrared absorption spectra of some 25 pyrrolizidine esters and their corresponding acids have been recorded in the 400–4000  $\text{cm}^{-1}$  range on Spectrophotometer Perkin-Elmer 521. A complete interpretation of the absorption peaks in terms of the types of vibrations has been provided and the characteristic frequencies isolated. It is found that the absorption peaks in the 740–760; 800–950; 960–980 and 1075–1130  $\text{cm}^{-1}$  could be assigned to ring deformation modes. Bands at around 610, 750, 965  $\text{cm}^{-1}$  can be used for identifying the presence of five membered nitrogen containing saturated ring. An inverse relationship has been observed between the C=O and C–O stretch frequencies.

## INTRODUCTION

PYRROLIZIDINE derivatives are well known for their physiological activity such as the anaesthetic and anti tumour action<sup>1</sup>. Some chemical investigations have been undertaken in the literature to establish the geometrical structure of these compounds. However no systematic work is reported on the structural study of these compounds using the spectroscopic methods, in particular the methods of vibrational spectroscopy. Evans and Wahr<sup>2</sup> have studied the infrared and Raman spectra of pyrrolidine molecule by giving assignments to various vibrations in the 300–3500  $\text{cm}^{-1}$  range. No such investigations for pyrrolizidine esters are reported in the literature. Culvenor *et al.*<sup>3,4</sup> have studied the carbonyl stretching frequencies of some pyrrolizidine alkaloids. Leisegang and Schuler<sup>5</sup> studied the existence of intermolecular hydrogen bond in platynecine and retronecine molecules. In the present communication, we have extended our studies to a detailed analysis of the infrared spectra of some pyrrolizidine esters in the 400–4000  $\text{cm}^{-1}$  range for finding out the spectra-structure relationship and the characteristic vibrations of the pyrrolizidine nucleus.

## EXPERIMENTAL

Platynecine, retronecine and their esters (III–XVII) were prepared by the method reported in our earlier communication<sup>6</sup>. The compounds were purified by column chromatography using alumina as adsorbent. The purity of the compounds was finally checked by t.l.c.

The spectra of most of the compounds were recorded in nujol mull because of their poor solubility. The spectra of platynecine (III) and retronecine (X) were recorded in KBr. pellets.

The spectra of acids constituting the esters were recorded for comparison purposes. All the spectra were recorded on infrared spectrophotometer Perkin-Elmer 521 in the 400–4000  $\text{cm}^{-1}$  range. The infrared spectra of pyrrolizidine (II) in carbon tetrachloride was kindly supplied to us by Dr. Björn Lunning of the University of Stockholm, Sweden. For pyrrolidine (I) the spectral data of Evans and Wahr has been used.

## RESULTS AND DISCUSSION

*OH-stretch vibrations.*—Platynecine and retronecine absorb at 3332 and 3315  $\text{cm}^{-1}$  respectively (Table I). Leisegang *et al.*<sup>4</sup> have interpreted this

TABLE I

*OH stretch and deformation vibration*

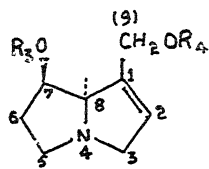
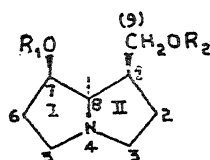
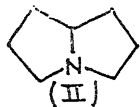
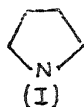
| Compound | $\nu_{\text{OH}}$<br>( $\text{cm}^{-1}$ ) | $\delta_{\text{CH}}$<br>( $\text{cm}^{-1}$ ) | $\gamma_{\text{OH}}$<br>( $\text{cm}^{-1}$ ) |
|----------|---|--|--|
| III      | 3332 (3340)*                              | 635, 655                                     | 1250   |
| IX       | 3410                                      | 632  | 1265   |
| X        | 3315 (3320)                               | 630, 665                                     | 1250   |
| XVI      | 3380                                      | 658  | 1240   |
| XVII     | 3400                                      | 638  | 1258   |

\* Figures given in parentheses correspond to the data of Leisegang *et al.* (Ref. 5).

band as indicative of the intermolecular hydrogen bond formation. The mono-substituted esters (IX, XVI, XVII) were found to absorb at 3410, 3380 and 3400  $\text{cm}^{-1}$  respectively. Thus the replacement of one of the OH groups in platynecine and retronecine by an ester group results in a higher frequency shift of the OH stretch vibration. This



increase in the frequency is perhaps caused by the reduction in the chain of molecules connected through hydrogen bonds.



- (III)  $R_1 = R_2 = H$   
 (IV)  $R_1 = R_2 = C_6H_5-CH=CH-CO$   
 (V)  $R_1 = R_2 = C_6H_5-CH_2-CO$   
 (VI)  $R_1 = R_2 = (CH_3)_2CH-CH_2-CO$   
 (VII)  $R_1 = R_2 = CH_3(CH_2)_{16}-CO$   
 (VIII)  $R_1 = R_2 = (CH_3)_2C=CH-CO$   
 (IX)  $R_1 = H, R_2 = p-CH_3O-C_6H_4-CO$   
 (X)  $R_2 = R_1 = H$   
 (XI)  $R_2 = R_1 = CH_3-(CH_2)_{15}-CO$   
 (XII)  $R_2 = R_1 = (CH_3)_2C=CH-CO$   
 (XIII)  $R_2 = R_1 = C_6H_5-CH=CH-CO$   
 (XIV)  $R_2 = R_1 = p-Cl-C_6H_4-CO$   
 (XV)  $R_2 = R_1 = CH_3-(CH=CH)_2-CO$   
 (XVI)  $R_2 = H, R_1 = CH_3-(CH=CH)_2-CO$   
 (XVII)  $R_2 = H, R_1 = p-CH_3O-C_6H_4-CO$

**CH<sub>2</sub> stretch vibrations.**—A number of absorption bands with medium to strong intensity were observed in 2800–3000 cm<sup>-1</sup> range which can be assigned to the CH<sub>2</sub> stretch vibrations. Pyrrolidine absorbs at 2817, 2865 and 2912, 2941 cm<sup>-1</sup>, due to symmetric and asymmetric CH<sub>2</sub> stretch vibrations<sup>2</sup> respectively. Pyrrolizidine, platynecine and retronecine were found to absorb at nearly the same frequencies as pyrrolidine (Table II). The

TABLE II  
CH<sub>2</sub> stretch vibrations

| Compound | Symmetric (cm <sup>-1</sup> )        | Asymmetric (cm <sup>-1</sup> )                    |
|----------|--------------------------------------|---|
| I        | 2817, 2865                           | 2912, 2941  |
| II       | 2810 (M),<br>2872 (M)                | 2905 (W),<br>2945 (S),<br>2965 (S)                |
| III      | 2815 (W),<br>2870 (S)                | 2925 (WM),<br>2940 (WM),<br>2965 (W),<br>2988 (W) |
| X        | 2825 (W),<br>2850 (WM),<br>2870 (WM) | 2915 (W),<br>2975 (W)                             |

band near 2810 cm<sup>-1</sup> has been assigned<sup>7,8</sup> to symmetric stretch of CH<sub>2</sub> attached to the nitrogen atom. Though it appears as a medium strong band in pyrrolizidine, it is only a weak band in platynecine and retronecine. In pyrrolizidine it is noticed that the intensity of the asymmetric stretch at about 2940 and 2960 cm<sup>-1</sup> is more than that of the symmetric stretch near 2870 cm<sup>-1</sup>. However reverse is the case in platynecine and retronecine where CH<sub>2</sub> symmetric stretch bands are more intense than the asymmetric stretch bands. This reversal in the intensity could be attributed to the presence of an electron donating group OH adjacent to the CH<sub>2</sub> in platynecine and retronecine.

**C—O and C=O stretch vibrations.**—Platynecine and retronecine absorb strongly at 1017 (VS), 1050 (S) cm<sup>-1</sup> and 1010 (S), 1040 (MS) cm<sup>-1</sup> respectively. The 1017 and 1010 cm<sup>-1</sup> absorption bands could be assigned<sup>9</sup> to C—O stretch of secondary alcohol (—COH) and 1050 and 1040 cm<sup>-1</sup> bands to C—O stretch of primary alcohol (—CH<sub>2</sub>OH). In the case of di-substituted esters, because of the presence of two more C—O bonds (due to ester group) four absorption bands are observed as listed in Table III; the additional bands appearing in the 1100–1300 cm<sup>-1</sup> range. In almost all the pyrrolizidine esters a band of medium to strong intensity corresponding to C=O stretch has been observed in the 1715–1740 cm<sup>-1</sup> range. It is found in a series of compounds that the C—O stretch frequency around 1270 cm<sup>-1</sup> decreases gradually while the C=O stretch frequency increases (Table III). A plot of C=O vs. C—O stretch frequencies is given in Fig. 1. It is evident from the figure that there exists a linear relationship between these frequencies which could be mathematically expressed as :

$$\nu_{C-O} = 4136 - 1.666 \nu_{C=O} \quad (1)$$

This relation however does not hold for isovalerates, and stearates, where the carbonyl group does not form a conjugated system. This behaviour is evident from the scattered points in Fig. 1. A similar relationship has been reported by Jones *et al.*<sup>10</sup> for acetoxy steroids where a carbonyl group enters into a conjugated system.

**CH<sub>2</sub> deformation vibrations.**—Evans *et al.*<sup>2</sup> have compared the infrared and Raman spectra of pyrrolidine molecule and have provided an interpretation to the absorption peaks in terms of the CH<sub>2</sub> bending, wagging, twisting and rocking vibrations. The frequencies of these vibrations are known<sup>9</sup> to be practically independent of the nature of the substituent. We have therefore provided an interpretation of the CH<sub>2</sub> vibrations of platynecine, retronecine and their esters in terms of similar vibrations of pyrrolidine molecule (Table IV). It follows from Table IV that CH<sub>2</sub> wagging

TABLE III  
*C—O and C=O stretch vibrations*

| Compound | $\nu_{C=O}$<br>( $\text{cm}^{-1}$ ) | $\nu_{C-O}$<br>( $\text{cm}^{-1}$ ) |      |       |              |
|----------|-------------------------------------|-------------------------------------|------|-------|--------------|
|          |                                     | (i)                                 | (ii) | (iii) | (iv)         |
| III      | ..                                  | 1017                                | 1050 | ..    | ..           |
| IV       | 1712, 1718                          | 1010                                | 1038 | 1176  | 1278 (1273)* |
| V        | 1740                                | 1040                                | 1085 | 1146  | 1243 (1237)  |
| VI       | 1738                                | 1050                                | 1080 | 1128  | 1290 (1240)  |
| VII      | 1740                                | 1030                                | 1085 | 1132  | 1300 (1237)  |
| VIII     | 1718, 1722                          | 1000                                | 1080 | 1140  | 1267 (1270)  |
| IX       | 1722, 1730                          | 1030                                | 1057 | ..    | 1260 (1258)  |
| X        | ..                                  | 1010                                | 1040 | ..    | ..           |
| XI       | 1740                                | 1050                                | 1080 | 1128  | 1290 (1237)  |
| XII      | 1733                                | 1015                                | 1090 | 1138  | 1242 (1240)  |
| XIII     | 1712                                | 1035                                | 1100 | 1172  | 1285 (1283)  |
| XIV      | 1720                                | 1018                                | 1018 | 1137  | 1270 (1270)  |
| XV       | 1730, 1735                          | 1002                                | 1078 | 1138  | 1255 (1253)  |
| XVI      | 1720                                | 1000                                | 1078 | ..    | 1270 (1270)  |
| XVII     | 1722                                | 1028                                | 1070 | ..    | 1271 (1267)  |

\* Numbers given in the parentheses correspond to the calculated values using eq. (1).

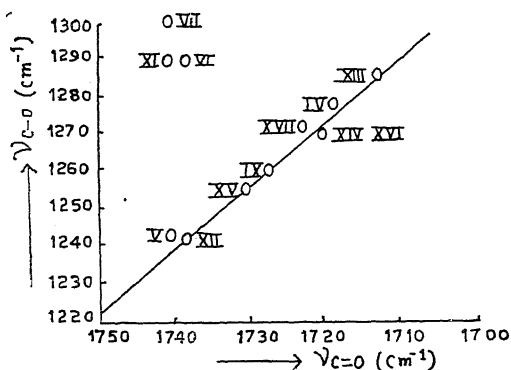


FIG. 1. C—O and C=O co-relationship.

vibrations appear in the ranges 970–990, 1102–1155, 1202–1250  $\text{cm}^{-1}$ , the twisting vibrations in the ranges 1150–1180, 1258–1299, 1337–1365  $\text{cm}^{-1}$  and the rocking vibrations in the ranges 785–852 and 1175–1208  $\text{cm}^{-1}$ . The absorption band at 1456  $\text{cm}^{-1}$  in pyrrolidine has been interpreted<sup>2</sup> as due to  $\text{CH}_2$  bending vibration. In pyrrolizidine this band appears

TABLE IV  
*CH<sub>3</sub> deformation vibrations*

| Compound | Wagging<br>( $\text{cm}^{-1}$ ) | Twisting<br>( $\text{cm}^{-1}$ ) | Rocking<br>( $\text{cm}^{-1}$ ) |
|----------|---------------------------------|----------------------------------|---------------------------------|
| I        | 980, 1100,<br>1225              | 1171, 1284,<br>1299, 1337        | 806, 1195                       |
| II       | 1155                            | 1175, 1290,<br>1352              | 845, 1208                       |
| III      | 985, 1225                       | 1170, 1275,<br>1285, 1355        | 832, 1192,<br>1207              |
| IV       | 990, 1210                       | 1180, 1278<br>1290, 1340         | 848, 1185                       |
| V        | 1108, 1225                      | 1170, 1350                       | 1180                            |
| VI       | 1122                            | 1165, 1270                       | ..                              |
| VII      | 1132                            | 1150, 1275,<br>1282              | 835                             |
| VIII     | 1120, 1228                      | 1160, 1265                       | 852                             |
| IX       | 965, 1120,<br>1225              | 1275                             | 810                             |
| X        | 990, 1102,<br>1202              | 1180, 1280,<br>1292, 1350        | 909, 1185                       |
| XI       | 970, 1120                       | 1290                             | 1175                            |
| XII      | ..                              | 1160, 1365                       | 1180                            |
| XIII     | 1125, 1210                      | 1160, 1285,<br>1340              | 840, 1185<br>(Sh)               |
| XIV      | 1225                            | 1270, 1364                       | 785, 1175<br>(W)                |
| XV       | 1250                            | 1270, 1290                       | 1190 (W)                        |
| XVI      | 1000, 1240                      | 1180, 1255                       | 835 (W),<br>1180 (W)            |
| XVII     | 1248                            | 1168, 1258,<br>1297              | 847                             |

at  $1452\text{ cm}^{-1}$ , while in cyclopentane and cyclohexane, it appears near  $1465\text{ cm}^{-1}$ . The lower frequency shift of this band in pyrrolidine and pyrrolizidine with respect to cyclic hydrocarbons could be attributed to the presence of nitrogen atom. In platynecine and retronecine each having two alcoholic groups, this band experiences a further lower frequency shift appearing at  $1432$  and  $1430\text{ cm}^{-1}$  respectively. However this band could not be separated out in the corresponding esters due to overlap by a  $\nu_{\text{C=O}}$  band.

*OH- deformation vibrations.*—The absorption bands in the range 1240–1265  $\text{cm}^{-1}$ . (Table I) have

TABLE V  
Ring deformation vibrations

| Compound | $\delta_{ccc}$<br>( $\text{cm}^{-1}$ ) | Ring modes<br>( $\text{cm}^{-1}$ ) |               |       |            |
|----------|--|------------------------------------|---------------|-------|------------|
|          |  | (i)                                | (ii)          | (iii) | (iv)       |
| I        | ..                                     | 612                                | 806, 902      | 980   | 1080, 1109 |
| II       | ..                                     | ..                                 | 890, 906      | ..    | 1078, 1098 |
| III      | 750                                    | 550, 602                           | 880, 882, 895 | 962   | 1088, 1112 |
| IV       | ..                                     | 615                                | 872           | 965   | 1085, 1120 |
| V        | 730                                    | 620                                | ..            | ..    | 1085, 1108 |
| VI       | 750                                    | 630                                | ..            | ..    | 1075       |
| VII      | 755                                    | ..                                 | 885           | 965   | ..         |
| VIII     | ..                                     | 622                                | ..            | 975   | 1077       |
| IX       | 745                                    | 530, 622                           | ..            | 965   | 1098       |
| X        | 748                                    | 545, 600                           | 868, 912      | 967   | 1080, 1102 |
| XI       | 755                                    | 630                                | ..            | 970   | 1075, 1120 |
| XII      | ..                                     | 610                                | 890           | ..    | 1100       |
| XIII     | ..                                     | 618                                | 870, 915      | 988   | 1085, 1097 |
| XIV      | 760                                    | 625                                | 850           | ..    | ..         |
| XV       | 745                                    | ..                                 | 875           | 960   | 1078, 1130 |
| XVI      | 760                                    | 570, 610                           | 870           | 965   | 1075, 1120 |
| XVII     | 740                                    | 565, 610                           | 840           | 960   | 1070, 1103 |

been assigned to the OH-deformation vibrations. An OH- out of plane deformation band of medium intensity is observed at about  $635\text{ cm}^{-1}$  in mono-substituted compounds. In platynecine and retronecine each having two OH groups, two such bands are observed in this region.

*Ring deformation modes.*—These are found to appear in the spectra of platynecine, retronecine and their esters, in the ranges 740–760, 800–915, 960–980 and  $1075\text{--}1130\text{ cm}^{-1}$  (Table V). The band of medium intensity in the  $740\text{--}760\text{ cm}^{-1}$  range

could be assigned to CCC deformation vibration between the two rings. In compounds containing a mono-substituted benzene ring, this band could not be separated from a similar band due to aromatic CH out of plane deformation vibration. The intensity of this band increases with the presence of an electron donating group at 7 and 9 positions. It is thus found that the intensity of this band is minimum in a di-substituted ester (XV), more in mono-substituted ester (XVI) and maximum in retronecine (X) where both the 7 and 9 positions are occupied by hydroxyl groups.

The  $902\text{ cm}^{-1}$  band in pyrrolizidine has been interpreted<sup>2</sup> as due to the ring breathing. Pyrrolizidine, platynecine and retronecine, each having two five membered rings, show two absorption bands appearing at 890, 906; 880, 895 and 868, 892  $\text{cm}^{-1}$  respectively. They are however not identifiable in all the corresponding pyrrolizidine esters. A band in the  $960\text{--}980\text{ cm}^{-1}$  range is found to be characteristic of all the platynecine and retronecine derivatives. In most of the cases it appears at about  $965\text{ cm}^{-1}$ . Another band near  $610\text{ cm}^{-1}$  also appears nearly at a constant position in all the esters. This band could also be attributed to the ring deformation mode.

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# FORMATION CONSTANTS OF THE CHELATES OF 2-HYDROXY-1-NAPHTHALIDENE-*p*-METHOXYANILINE WITH SOME DIVALENT METAL IONS

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## ABSTRACT

Potentiometric studies have been carried on the metal complexes of  $\text{Co}^{+2}$ ,  $\text{Ni}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Zn}^{+2}$ ,  $\text{Cd}^{+2}$  and  $\text{Mg}^{+2}$  with 2-hydroxy-1-naphthalidene-*p*-methoxyaniline. The dissociation constants ( $\text{pK}_1$  and  $\text{pK}_2$ ) of the reagent and the formation constants of its metal complexes have been determined by Bjerrum's method at  $30 \pm 0.1^\circ \text{C}$ . The order of stability is found to be  $\text{Cu} > \text{Ni} > \text{Zn} > \text{Co} > \text{Cd} > \text{Mg}$ .

**L**ITERATURE survey indicates that no systematic study on the stabilities of 2-hydroxy-1-naphthalidene-*p*-methoxyaniline and its metal chelates with bivalent metal ions has been carried out. In the present communication, the successive stability constants of the complexes of 2-hydroxy-1-naphthalidene-*p*-methoxyaniline with various divalent metal ions have been determined potentiometrically following the Calvin-Bjerrum pH titration technique as adopted by Irving and Rossotti<sup>1</sup>.

## EXPERIMENTAL

The Corning Model 12, a precision research pH meter was employed throughout the work for pH determinations. The ligand was synthesised and repeatedly crystallised to get an analytically pure sample (m.p.  $109^\circ \text{C}$ )<sup>2</sup>.

The medium of titration was dioxan-water mixture containing 75% (v/v) of dioxan. The dioxan used for the experiments was purified by the method described by Vogel<sup>3</sup>. Conductivity water was used throughout the investigation. Sodium perchlorate was added to maintain constant ionic strength (0.1 M). The titrations were carried out in an inert atmosphere by bubbling the nitrogen gas through the solutions. All the metal perchlorate solutions were standardised complexometrically<sup>4</sup> by E.D.T.A. titrations. All measurements were made at  $30 \pm 0.1^\circ \text{C}$ .

The following solutions were titrated potentiometrically against standard carbonate free sodium hydroxide (1.092 M) solution, keeping the total volume 40 ml.

- (i) 5 ml of (0.16 M)  $\text{HC}'\text{O}_4 + 5$  ml of (0.64 M)  $\text{NaClO}_4 + 30$  ml of dioxan.
- (ii) 5 ml of (0.16 M)  $\text{HClO}_4 + 5$  ml of (0.64 M)  $\text{NaClO}_4 +$  requisite amount of the reagent accurately weighed to give 0.01 M reagent concentration in the final solution + 30 ml of dioxan.
- (iii) 5 ml of (0.64 M)  $\text{NaClO}_4 + 5$  ml of (0.024 M) metal salt solution in (0.16 M)  $\text{HClO}_4 +$  requisite amount of the reagent

accurately weighed to give 0.01 M, 0.05 M and 0.03 M reagent concentration in the final solution in the case of ( $\text{Cu}^{+2}$ ,  $\text{Co}^{+2}$ ,  $\text{Ni}^{+2}$ ,  $\text{Zn}^{+2}$ );  $\text{Mg}^{+2}$  and  $\text{Cd}^{+2}$  respectively + 30 ml of dioxan.

All titrations were performed in duplicate to test for reproducibility.

The experimental method of Irving and Rossotti<sup>1</sup> was applied to find the values of  $\bar{n}$  and pL.

## RESULTS AND DISCUSSION

In the ligand, it is the chelated phenolic 'OH' group which takes part in the complex formation and the proton is replaced from it by metal ions during the formation of metal chelates. Since only one proton per ligand molecule is liberated during complexation, 'Y' the number of dissociable protons attached per ligand molecule is equal to one.

From the titration curves using the solutions (i) and (ii)  $\bar{n}_A$  values at various 'B' values (pH meter readings) were calculated and the curve between 'B' and the corresponding  $\bar{n}_A$  values was plotted (Fig. 1). The formation curve extends over a range  $0.85 < \bar{n}_A < 1.8$  and is wavelike. This indicates the formation of the species HL and  $\text{H}_2\text{L}^+$ , i.e., the protonated nitrogen and the phenolic hydrogen are completely dissociable in steps separable.

Three methods known as half integral, graphical and least square<sup>7</sup> are suitably employed here to calculate the stability constants.

In the half integral method, the values of pL at  $\bar{n} = 0.5$  and  $\bar{n} = 1.5$  were taken as  $\log K_1$  and  $\log K_2$  respectively from the formation curves extending over a range of  $0 < \bar{n} < 2$ . In the cases where stepwise formation of complexes is indicated by the flattening of the formation curves at integer values of  $\bar{n}_A$  or  $\bar{n}$ , the different portions of 1:1 and 1:2 complexes were treated separately and the respective  $\text{pK}'$  and  $\log K$  values were determined by graphical method from the linear plots of,  $\log \frac{\bar{n}_A}{(1 - \bar{n}_A)}$  or  $\log \frac{(2 - \bar{n}_A)}{(1 - \bar{n}_A)}$  versus 'B' (pH meter

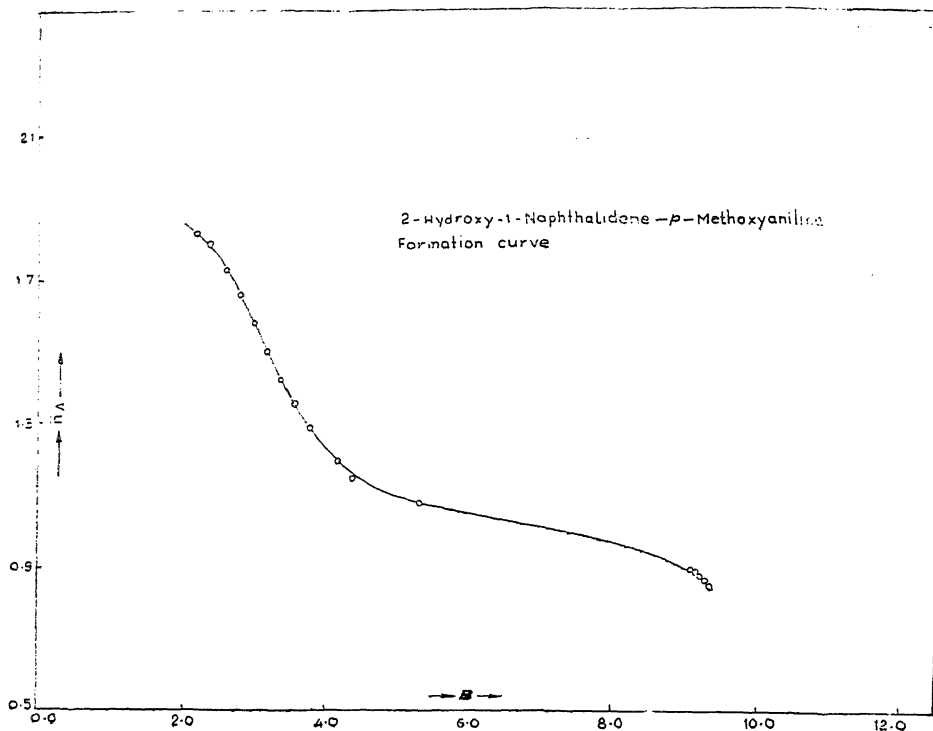


FIG. 1

readings) and  $\log \bar{n}/(1 - \bar{n})$  or  $\log \frac{(2 - \bar{n})}{(1 - \bar{n})}$  versus  $pL$  as the case may be by employing the relation

$$\bar{n}_A + (\bar{n}_A - 1) \cdot pK_2^H \cdot \frac{1}{\text{antilog } B} = 0$$

and

$$(\bar{n}_A - 1) - (2 - \bar{n}_A) pK_2^H \cdot \frac{1}{\text{antilog } B} = 0$$

and

$$\log K = \log \frac{\bar{n} - (i - 1)}{(i - \bar{n})} + pL$$

In the cases where the formation curves are incomplete in the sense that they do not reach the value of  $\bar{n} = 1.5$  and in the cases in which the formation curves are not wavelike indicating that the formation of the second complex starts before the completion of the 1:1 complex,  $\log K$  values are calculated by the least square method:

$$\bar{n} = \frac{K_1(L) - 2K_1K_2(L)^2 + \dots - NK_1K_2 \dots K_N(L)^N}{1 + K_1(L) + K_1K_2(L)^2 + \dots + K_1K_2 \dots K_N(L)^N}$$

This equation for 1:1 and 1:2 complexes can be written in the linear form as:

$$\frac{\bar{n}}{(\bar{n} - 1)L} = \frac{(2 - \bar{n})}{(1 - \bar{n})} \cdot (L) K_1K_2 - K_1$$

knowing the quantities  $\frac{\bar{n}}{(\bar{n} - 1)(L)}$  and  $\frac{(2 - \bar{n})}{(\bar{n} - 1)} \cdot (L)$

For each point on the formation curve the above equation is solved by the least square method to get the values of  $K_1$  and  $K_2$ . The value of  $pK_2^H$  only could be evaluated from half integral point at  $\bar{n}_A = 1.5$ . The value of  $pK_1^H$  could not be found by half integral methods. It was however determined by least square method.

A plot of  $\log \left[ \frac{2 - \bar{n}_A}{1 - \bar{n}_A} \right]$  against  $B$  was also drawn but is not included here for economy of space. From this curve the value of practical  $pK_2^H$  was evaluated. The two values agree quite well.

From the titration curves of the solutions (ii) and (iii)  $\bar{n}$  and  $pL$  values were calculated. The  $\bar{n}$  values were plotted against the corresponding  $pL$  values to get the formation curves of the metal complexation equilibria (Fig. 2). From these formation curves the values of stability constants  $\log K_1$  were determined which correspond to the  $pL$  values at  $\bar{n} = 0.5$ . The least square method was applied to calculate  $\log K_1$  and  $\log K_2$  in the case of  $\text{Co}^{+2}$  and  $\text{Mg}^{+2}$ . The most representative values are recorded in Table I.

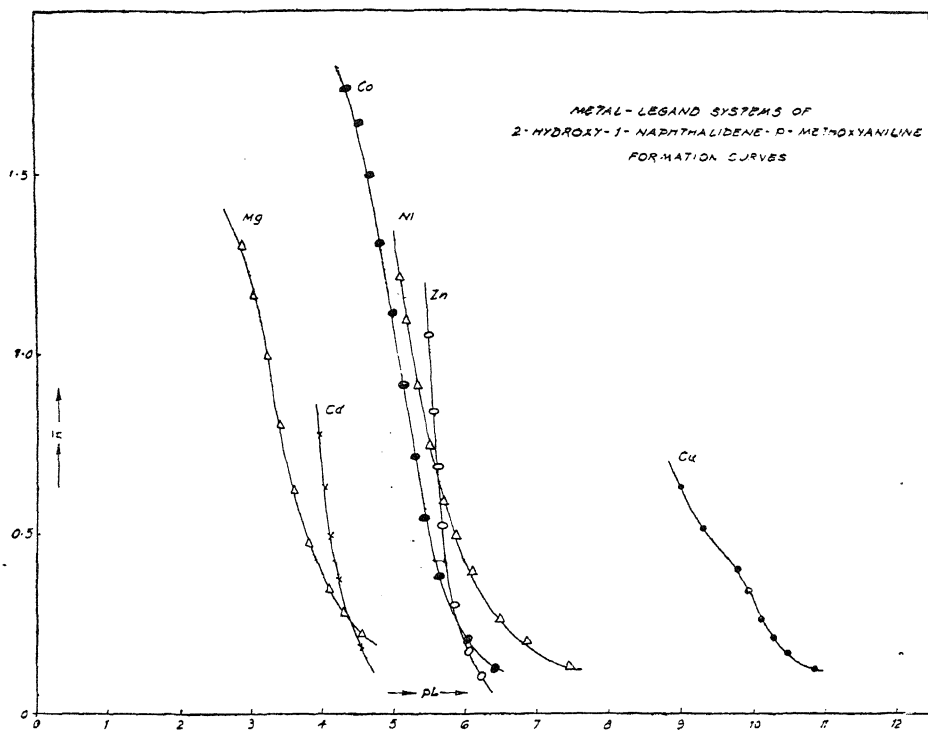


FIG. 2

TABLE I

Stepwise formation constants of various complexes

| Metal ions         | H <sup>+</sup> | Cu <sup>+2</sup> | Ni <sup>+2</sup> | Co <sup>+2</sup> | Zn <sup>+2</sup> | Cd <sup>+2</sup> | Mg <sup>+2</sup> |
|--------------------|----------------|------------------|------------------|------------------|------------------|------------------|------------------|
| log K <sub>1</sub> | 10.00          | 9.35             | 5.84             | 5.37             | 5.66             | 4.10             | 3.77             |
| log K <sub>2</sub> | 3.22           | ..               | ..               | 4.80             | ..               | ..               | 2.67             |

The order of stability of bivalent metal chelates was Cu > Ni > Zn > Co > Cd > Mg.

The order in the case of Zn<sup>+2</sup> complex is reversed with respect to Co<sup>+2</sup> as compared to that observed by Maley and Mellor<sup>5</sup>. The reversal of the order in the case of cobalt and zinc may be attributed to the closeness of the values in the present investigation.

A parallelism between log K and second ionisation potential (I.P.) is also observed in the present case as has been suggested by Irving and Williams<sup>6</sup>. This is verified graphically by plotting the (stability

constants) formation constants and the ionisation potentials as a function of the atomic number.

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# INFLUENCE OF VARIOUS HOSTS ON THE DEVELOPMENT AND REPRODUCTION OF THE PUPAL PARASITE, *TETRASTICHUS ISRAELI* M. AND K. (EULOPHIDAE: HYMENOPTERA)

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**F**OR the successful utilisation of natural enemies in the control of several pests of crop plants, the knowledge of their host specificity or polyphagy, host selection, prolificacy, etc., is of vital importance. This information is particularly useful in the mass multiplication of the parasite on alternate hosts in the laboratory and subsequent release in the field. With this in view, studies were made on an eulophid pupal parasite, *Tetrastichus israeli* M. and K., which is mass produced in South India for the control of coconut caterpillar, *Nephantis serinopa* M. In testing the suitability of hosts, the effect of eight species of lepidopterous insects, viz., *N. serinopa*, *Piusia peponis* F., *Margaronia indica* S., *Corcyra cephalonica* St., *Spodoptera litura* (F.), *Chilo infuscatellus* S., *Phycodes radiata* O. and *Orthaga exvinacea* W. on the development and reproduction of the parasite has been observed.

Five gravid females of equal size and age were inoculated in a tube and fresh *C. cephalonica* pupa was supplied. The parasites that emerged from the pupa were used to parasitise different hosts for studying the development and fecundity of *T. israeli*.

**Effect on development:** Among the hosts studied, *N. serinopa* and *M. indica* hastened the parasite development in 13.2 and 13.8 days respectively. Delayed development was observed in the pupa of *O. exvinacea* (16.2 days), and the other hosts were medium in their effect (Table I). Similar influence of host on the rate of development of parasite was noted in *Microbracon gelechiae* Ashm. on different hosts<sup>1</sup> and in aphid parasites<sup>2,3</sup>. Arthur and Wylie<sup>4</sup> proved that the rate of development was much longer in larger hosts than in smaller ones. These results have been confirmed in the present studies: In the pupa of *P. peponis*, *S. litura*, *O. exvinacea* and *C. infuscatellus*, where the quantity of food is more, the total embryonic and post-embryonic development period was prolonged. House<sup>5</sup> noted reduction in the rate of growth of the tachinid, *Agria affinis* (Fall.) when the growth-promoting amino acids like glycine, serine, alanine and tyrosine were deleted from the artificial diet. In the pupa of *N. serinopa* and *M. indica*, the above-mentioned amino acids are abundantly present (Table II). The ratio of these

amino acids to the total content was 0.3 in *M. indica* and *N. serinopa* as against 0.1 to 0.2 in *P. peponis* and *C. cephalonica*, and 0.01 in *O. exvinacea*, where the total development period of the parasite was prolonged.

House and Barlow<sup>6</sup> reported the importance of concentration of potassium in the growth of the tachinid, *A. affinis* as it helped in the rapidity of growth. A low content of 1.9% of K is found in *O. exvinacea* where the development was markedly delayed (Table II). The high content found in *N. serinopa*, *M. indica* and *P. peponis* is apparently favourable for the development of the parasite. However, the high content observed in *S. litura* is somewhat erratic in that it has not favoured the parasite development probably due to the total absence of growth-promoting amino acids. The same authors have also reported that K in high concentration was toxic to the parasite. This is in accordance with the present findings on *C. cephalonica* and the highest K content present in this host may be detrimental to the parasite. Chen<sup>7</sup> reported about the importance of arginine, cystine, glycine, proline, tryptophan, tyrosine and phenylalanine either for moulting, differentiation, pupation or adult emergence. Most of these are present in the favoured hosts (Table II).

**Effect on fecundity.**—Increased number of parasites emerged from the pupa of *P. peponis* (162.6) and *S. litura* (153.0) which were the most favourable hosts. The other hosts like *C. cephalonica*, *M. indica*, *N. serinopa* and *P. radiata* gave rise to minimum number of parasites ranging from 33.2 to 38.8 (Table I). There was a positive relationship between the weight of host pupae and the number of parasites emerged (Fig. 1). This is in accordance with the report of Rao *et al.*<sup>8</sup> in *Bracon brevicornis* W. The process of oviposition was reported to last more time when the parasite attacked big sized aphids and so more eggs could be laid<sup>9,10</sup>. In the present observations, *T. israeli* took more time when ovipositing in *P. peponis* pupae which are big sized.

**Effect on sex ratio and adult longevity.**—Greater proportion of females was noted among the parasites emerged from *N. serinopa*, *M. indica* and *C. cepha-*

TABLE I

Effect of various hosts on development period, fecundity, sex ratio, adult longevity and size of *Tetrastichus israeli*  
(Mean of 5 observations)

| Hosts                      |    | Development period (days) | Fecundity | Sex ratio ♂/♀ | Longevity (days) |      | Size (mm)   |             |
|----------------------------|----|---------------------------|-----------|---------------|------------------|------|-------------|-------------|
|                            |    |                           |           |               | ♂                | ♀    | ♂           | ♀           |
| <i>Nephantis serinopa</i>  | .. | 13.2                      | 35.0      | 0.07          | 2.0              | 15.2 | 1.44 × 0.41 | 1.70 × 0.46 |
| <i>Plusia peponis</i>      | .. | 14.2                      | 162.6     | 0.11          | 2.4              | 10.0 | 1.51 × 0.38 | 1.94 × 0.48 |
| <i>Margaronia indica</i>   | .. | 13.8                      | 37.0      | 0.08          | 2.0              | 9.0  | 1.27 × 0.37 | 1.56 × 0.43 |
| <i>Chilo infuscatellus</i> | .. | 15.0                      | 94.8      | 0.19          | 1.3              | 6.0  | 1.40 × 0.36 | 1.54 × 0.39 |
| <i>Phycodes radiata</i>    | .. | 14.8                      | 33.2      | 0.16          | 3.4              | 6.6  | 1.67 × 0.40 | 2.00 × 0.58 |
| <i>Orthaga exvinacea</i>   | .. | 16.2                      | 102.2     | 0.15          | 1.5              | 6.6  | 1.26 × 0.37 | 1.61 × 0.42 |
| <i>Spodoptera litura</i>   | .. | 15.6                      | 153.0     | 0.18          | 1.3              | 6.2  | 1.46 × 0.42 | 1.94 × 0.51 |
| <i>Corecya cephalonica</i> | .. | 14.4                      | 38.8      | 0.08          | 1.5              | 8.2  | 1.03 × 0.28 | 1.44 × 0.37 |
| Mean                       | .. | 14.7                      | 82.1      | 0.13          | 1.9              | 8.5  | 1.38 × 0.37 | 1.72 × 0.46 |
| C.D. (P=0.05)              | .. | 0.9                       | 25.6      | 0.03          | 0.5              | 1.3  | 0.12 0.03   | 0.14 0.05   |

\* Body length × breadth at thorax.

TABLE II

Amino acid and potassium contents in various hosts of *Tetrastichus israeli*

| Content                   | <i>N. serinopa</i> | <i>P. peponis</i> | <i>M. indica</i> | <i>C. cephalonica</i> | <i>S. litura</i> | <i>O. exvinacea</i> |
|---------------------------|--------------------|-------------------|------------------|-----------------------|------------------|---------------------|
| AMINO ACIDS<br>(µg/pupa)  |                    |                   |                  |                       |                  |                     |
| Aspartic acid             | ..                 | ..                | ..               | 451.7                 | ..               | ..                  |
| Glutamic acid             | ..                 | 77.6              | 202.3            | 67.8                  | 16.7             | 115.2               |
| Glycine and/or Serine     | ..                 | 15.9              | 76.0             | 24.5                  | 28.2             | ..                  |
| Lysine                    | ..                 | 29.1              | ..               | ..                    | ..               | ..                  |
| Glutamine                 | ..                 | 29.1              | ..               | ..                    | ..               | ..                  |
| Threonine                 | ..                 | 17.4              | ..               | 15.4                  | 8.8              | ..                  |
| Alanine                   | ..                 | 16.8              | ..               | 29.8                  | ..               | 6.7                 |
| Tyrosine                  | ..                 | 77.6              | 259.7            | 83.8                  | ..               | ..                  |
| Histidine                 | ..                 | 58.1              | ..               | 42.8                  | 55.7             | ..                  |
| Arginine                  | ..                 | ..                | ..               | 29.1                  | ..               | ..                  |
| Proline                   | ..                 | 19.0              | ..               | Trace                 | ..               | 52.4                |
| Methionine                | ..                 | 63.6              | 138.3            | 18.8                  | ..               | 237.0               |
| Valine                    | ..                 | 29.9              | 27.3             | 42.3                  | 11.1             | 118.2               |
| Leucine and/or isoleucine | ..                 | ..                | 875.0            | 126.7                 | 24.2             | 120.0               |
| Total No. of amino acids  | 12                 | 8                 | 13               | 9                     | 5                | 8                   |
| Total quantity            | ..                 | 434.1             | 1578.6           | 481.0                 | 596.4            | 590.4               |
| Potassium %               | ..                 | 2.9               | 2.1              | 2.8                   | 3.3              | 2.9                 |
|                           |                    |                   |                  |                       |                  | 1.9                 |



*lonica* (Table I). This influence of hosts confirm the earlier observations of Flanders<sup>11</sup> that fertilised eggs were laid in suitable hosts and unfertilized eggs in unsuitable hosts resulting in more of males. The male parasites reared from *C. infuscatellus* and *S. litura* lived shorter (1.3 days), while the female longevity was upto 15.2 days in the case of parasites from *O. exvinacea*, *P. radiata*, *S. litura* and *C. infuscatellus* (Table I).

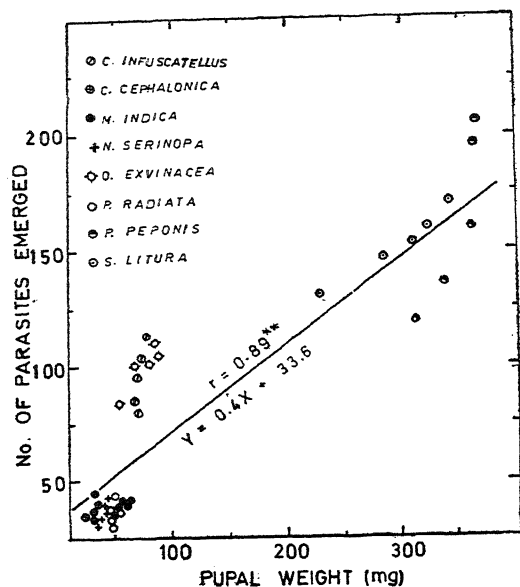


FIG. 1. Correlation between weight of pupa and total number of parasites emerged from different hosts.

**Effect on size of the parasite.**—Influence of host on the size of parasite was seen in all the cases. Larger females were noted from the pupae of *P. peponis*, *S. litura* and *Orthaga exvinacea* as against the smallest size from *C. cephalonica*; *N. serinopa* and *M. indica* produced females of medium size (Table I). Fecundity of the parasite seems to be influenced by a host species by affecting the size of the parasite as observed by Hafez<sup>12</sup>

in the case of *Aphidius rapae* (Curtis), a parasite of the cabbage aphid.

The amino acids related to the morphogenic events during insect development were arginine, histidine, threonine, leucine, isoleucine and valine. They were found to be essential for insect growth<sup>13,14</sup>. The total quantity of these amino acids was more in the case of *P. peponis*, *S. litura* and *O. exvinacea* (Table II) from which bigger parasites emerged. The size was correspondingly reduced with the reduction of these amino acids in the host pupa. The least content in *C. cephalonica* was associated with the smallest size of the parasites emerged from this host. The length of abdomen was positively correlated with the total number of parasites emerged from *C. cephalonica* ( $r = 0.87^{**}$ ;  $Y = 105.1X - 41.3$ ) and so the fecundity would be increasing with the increase in abdomen size.

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## LETTERS TO THE EDITOR

### SPECTROGRAPHIC DETERMINATION OF TRACE IMPURITIES IN STRONTIUM CARBONATE

IN electronics industry, strontium carbonate is required in a highly purified form for cathode spraying and thus it becomes necessary to analyse  $\text{SrCO}_3$  for trace impurities. A spectrographic method is, therefore, developed for this purpose. A literature survey did not show any suitable method for the analysis of  $\text{SrCO}_3$  as matrix for impurities in parts per million (ppm) range. The present method is simple, straight-forward and very convenient for routine and rapid analysis of Al, Ba, Ca, Cd, Cu, Fe, Mg, Na, Pb and Zn in parts per million range.

Standards are prepared synthetically by dry mixing the specpure grade oxides of above-mentioned impurities with specpure  $\text{SrCO}_3$ . All compounds used are supplied by Johnson Matthey and Co. The sample (standard) is thoroughly ground with equal quantity of specpure U.C.C. (Ultra Carbon Corporation) graphite containing 100 ppm of specpure  $\text{Cr}_2\text{O}_3$  in order to use Cr as internal standard element. 50 mg of this mixture is loaded in the cavity of 6.15 mm diameter preformed 100-L U.C.C. graphite electrode and warmed under an infrared lamp for 10 minutes to drive away traces of moisture. The sample as anode is excited at 15 amps. d.c. for a period of 40 seconds. The spectrum is then photographed on Ilford R-40 emulsion and in the region 2750 Å–3375 Å in the first order of a 1,200 lines/mm grating blazed at 3,300 Å and employing Jaco 3.4 meter Ebert Spectrograph.

In order to improve the sensitivity of the method and to avoid selective volatilisation of the impurities, the experiments were conducted with different ratios (4 : 1) and (1 : 1) of sample to graphite and at 15 and 10 amperes d.c. excitation. It is found that the ratio of 1 : 1 at 15 amperes d.c. improved the sensitivity and volatilisation of the impurities. In addition Bi 2897.97 Å and Cr 2835.6 Å were tried as internal standard elements for volatile and non-volatile impurities. It is observed experimentally that the average standard deviation calculated with respect to these internal standard elements are of the same order. Consequently Cr 2835.6 Å is used as internal standard line. Other analytical details are given in Table I. The working curves are plotted for a set of standards

TABLE I  
*Analytical data for determination of impurities in  
strontium carbonate*

| Sl. No. | Analytical line<br>(Å) | Concentration<br>Range<br>(ppm) | Precision<br>± % |
|---------|------------------------|---------------------------------|------------------|
| 1.      | Al 3082.2              | 1–20                            | 13               |
| 2.      | Ba 3071.6              | 50–200                          | 11               |
| 3.      | Ca 3179.3              | 20–200                          | 12               |
| 4.      | Cd 3261.1              | 10–200                          | 10               |
| 5.      | Cu 3274.0              | 1–20                            | 13               |
| 6.      | Fe 2966.9              | 1–20                            | 14               |
| 7.      | Mg 2779.8              | 1–20                            | 10               |
| 8.      | *Na 3302.3             | 50–200                          | ..               |
| 9.      | Pb 2833.1              | 1–20                            | 16               |
| 10.     | *Zn 3303.0             | 50–200                          | ..               |

\* These elements are estimated semiquantitatively.  
Cr 2835.6 is used as an internal standard line for above elements.

for different impurities. The curves are linear and practically parallel to each other. Since the blank  $\text{SrCO}_3$  contains residual amounts of Fe = 2 ppm and Mg = 2 ppm, the working curves are plotted by applying correction by method of trial additions.

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### POLYPHENOLS OF *SOLANUM TORVUM*

ALTHOUGH many investigators have reported the presence of alkaloids<sup>1-3</sup> and steroids<sup>4,5</sup> isolation and identification of polyphenols from *Solanum torvum* Swartz (Family : Solanaceae, Tamil name : Sundaikai) has not been reported. Hence it is thought interesting to study the polyphenolic system in the fruits of *S. torvum* that serves as good

substrate for the polyphenoloxidase present in the same plant.

*Isolation of phenolic acids.*—The fresh fruits (2 kg) were stirred for a few minutes with half their volume of hot water (80° C) to inactivate the polyphenoloxidase. The fruits were then blended with aqueous methanol (80% v/v; 8 lit.). Chlorophyll and other fatty materials were removed by shaking the methanol extract with petroleum ether (b.p. 40–60° C). The phenols were precipitated with saturated neutral lead acetate solution. The precipitate thus obtained was centrifuged and the moist precipitate was stirred repeatedly with small quantities of Amberlite IR-120 (H<sup>+</sup> form) till a clear solution was obtained. The solution was filtered free of the resin, concentrated under vacuum and extracted repeatedly with ether followed by ethylacetate. Thin layer chromatography on silica gel layers using the solvent system chloroform-ethyl acetate-formic acid (5 : 4 : 1 v/v) showed that the ether extract mainly contained three compounds with  $R_f$  values 0.07, 0.18 and 0.60 and ethylacetate extract, two only, those with  $R_f$  values 0.07 and 0.18.

Quantitative separation of the three major compounds was carried out by column chromatography using polyamide (Ultramid B 3, BASF) as adsorbent. The column was equilibrated with benzene. The mixture of phenols was adsorbed on a little polyamide and transferred to the top of the column. Elution was carried out with benzene-chloroform mixture (50 : 50 v/v) with increasing concentrations of methanol. Initially the compound with  $R_f$  0.6 was eluted with 5% methanol in 50 : 50 benzene-chloroform mixture (Band I). With 15% methanol in 50 : 50 benzene-chloroform mixture, the compound with  $R_f$  0.18 was eluted (Band II). In the later stage, the compound with  $R_f$  0.07 (Band III) was eluted along with traces of Band II.

Band I, crystallized from methanol, was found to melt at 210–212° C. Mixed m.p., and superimposability of I.R. spectra with an authentic sample (Koch-Light Laboratory) showed that Band I is caffeic acid.

#### Analysis

Found C, 60.22, H, 4.59 ;

Calculated for  $C_9H_8O_4$  : C, 60.02 ; H, 4.67%.

Band II could not be crystallized but was obtained as an amorphous powder by precipitation with butylacetate-chloroform mixture (1 : 10 v/v). This compound was characterized as isochlorogenic acid by comparison with authentic sample isolated from coffee beans following the method of Barnes *et al.*<sup>6</sup> Though isochlorogenic acid behaves as a single

compound during isolation or in chromatography in non-aqueous solvents, it was resolved into 4, 5-, 3, 4- and 3, 5-dicafeoylquinic acids (designated as isochlorogenic acid A, B and C) by Corse *et al.*<sup>7</sup> by counter-current distribution. A 4th compound which appeared to be a mixture of the 3'-methylethers of 3, 5-dicafeoylquinic acid was also obtained by them. Earlier Lentner and Deatherage<sup>8</sup> also reported the resolution of isochlorogenic acid into four spots by paper chromatography. The resolution of isochlorogenic acid into four spots was also confirmed in the present study using thin layer chromatography over silica gel layers in the solvent system chloroform-ethylacetate-formic acid (5 : 4 : 1 v/v) ( $R_f$  0.41, 0.35, 0.32 and 0.28). All these observations indicated the presence of dicafeoylquinic acids which were supported by elementary analysis.

#### Analysis

Found C, 55.74 ; H, 4.98 ;

Calculated for  $C_{25}H_{26}O_8 \cdot H_2O$  : C, 56.17, H, 4.90%.

Band III was purified first by fractional precipitation with methanol-ethylacetate-petroleum ether mixture (1 : 3 : 10 v/v) and then by repeated crystallization with water, m.p. 208–210° C. Co-chromatography, mixed m.p. and superimposability of I.R. spectra with an authentic sample isolated from coffee beans confirmed its identity as chlorogenic acid.

The ethylacetate fraction containing only chlorogenic and isochlorogenic acids was partitioned between butylacetate and phosphate buffer<sup>6</sup> (pH 5.2, 2 M). The butylacetate fraction was found to contain isochlorogenic acid.

The aqueous solution was extracted with *n*-butanol and the extract was chromatographed on a polyamide column as described earlier. Elution with 15% methanol in benzene-chloroform (50 : 50 v/v) mixture yielded chlorogenic acid.

Neochlorogenic acid was eluted with 25% methanol in benzene-chloroform (50 : 50 v/v) mixture and it was identified by cochromatography with an authentic sample isolated from coffee beans m.p. 204–206° C.

The yield of acids : Caffeic acid, 100 mg ; Chlorogenic acid, 1.09 g ; Neochlorogenic acid, traces and Isochlorogenic acid, 150 mg.

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# A NOVEL AND RAPID METHOD FOR *IN VITRO* TESTING OF ANTIAMOEBCIC AGENTS AGAINST AEROBIC AND ANAEROBIC AMOEBAE GROWING AXENICALLY OR WITH BACTERIA

VARIOUS methods have been used in the past for screening antiamoebic compounds *in vitro* against anaerobic *E. histolytica*, *E. invadens*, etc., and aerobic free-living amoebae of pathogenic and non-pathogenic types<sup>3-9</sup>. Many conflicting results as to the efficacy of a given drug have been reported<sup>1-2</sup>. For the first time, a simple rapid and reliable cavity slide method of *in vitro* drug testing, against aerobic and anaerobic amoebae growing axenically or with bacteria, has been described in this communication.

**Experimental.**—Trophozoites of 24 to 48 hr old cultures growing in modified Boeck and Drbohlav medium<sup>10</sup> with mixed bacterial flora and rice starch were pooled and their number counted by haemocytometer. 1,000 and 2,000 trophozoites in 0.2 ml were added to hallow ground slides (Fig. 1). Each cavity was filled with 0.7 ml of liquid overlay of fresh B and D medium and a small quantity of sterile rice starch (Difco) was added. The cavities were covered with coverslips and put in moist chamber in petri dishes and incubated at 37° C for  $\frac{1}{2}$  to 1 hr for amoebae to become motile. 0.1 ml of the appropriate drug concentration was thin added taking care that no air bubble remained in the cavity. The edges of the cover slips were sealed with paraffin wax. In control 0.1 ml of distilled water was added.

In the case of drug screening against axenically grown *E. histolytica*, trophozoites of 48 to 72 hr old cultures growing in modified Diamond's medium<sup>11-12</sup> were collected by centrifugation following the method of Das and Prasad<sup>13</sup>. 0.2 ml ino-

culum containing about 2,000 amoebae was put into cavity slide and filled with fresh medium (0.8 ml) containing the required concentration of the drug dilutions. The cavity was covered with cover slips, and the edges of cover slip were sealed with paraffin wax. The slides were put in moist chamber at 37° C (Fig. 1). Observations were taken after 6, 18, 24, 48 and 72 hr under inverted microscope to find out whether the amoebae were dead or alive. In doubtful cases subcultures were made in fresh culture medium. Petri dishes served as moist chamber were sealed from outside by adhesive tape to avoid contamination. In the case of the control, no drug was added. Duplicate sets were run for each drug dilution.

## Cavity Slides Inside Moist Chamber

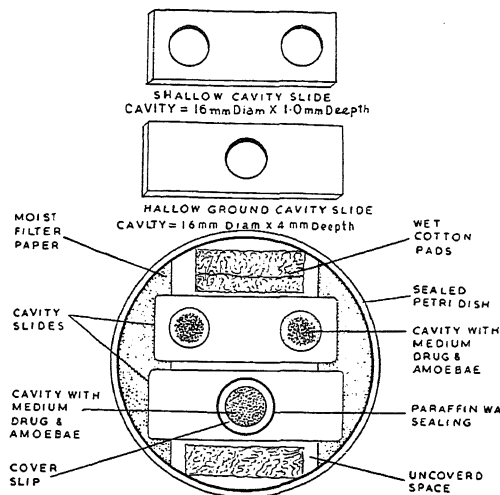


FIG. 1. Diagrammatic presentation of cavity slide method for antiamoebic drug screening.

In the case of the aerobic free-living amoebae, trophozoites from 24 to 48 hr old cultures growing on non-nutrient agar and bacteria (14) were washed with sterile distilled water by centrifugation and suspended in water containing *Escherichia coli* bacteria. The number of amoebae was determined by haemocytometer. The suspension (0.1 ml) containing about 1000 trophozoites was added to a cavity slide. (16 mm in diameter and 1.0 cm deep). The slides were put in moist chamber in Petri dishes and incubated at 25° C or 37° C for 30 minutes for amoebae to become motile. Drug (0.1 ml) was then added to a slide and slides were incubated for 6, 18, 24 and 48 hr. In the control 0.1 ml distilled water was added. Microscopic examination was done to find out whether the amoebae were dead or alive. In doubtful cases subcultures were made on non-nutrient agar plates

TABLE I

*In vitro* amoebicidal effect of drugs ( $\mu\text{g/ml}$ ) tested against both aerobic pathogenic *Naegleria aerobia* and *Hartmannella culbertsoni* and anaerobic *Entamoeba histolytica* growing exenically or with bacteria (xenic)

|                           | <i>E. histolytica</i><br>(Xenic) | <i>E. histolytica</i><br>(Axenic) | <i>N. aerobia</i><br>(Monoxenic) | <i>N. aerobia</i><br>(Axenic) | <i>H. culbertsoni</i><br>(Monoxenic) | <i>H. culbertsoni</i><br>(Axenic) |
|---------------------------|----------------------------------|-----------------------------------|----------------------------------|-------------------------------|--------------------------------------|-----------------------------------|
| Emetine hydrochloride     | 15.0                             | 7.5                               | 15.0                             | ..                            | 250.0                                | ..                                |
| Metronidazole (Flagyle)   | 4.0                              | 1.0                               | NA                               | ..                            | NA                                   | ..                                |
| 5-Fluorocytosine          | NA                               | NA                                | 40.0                             | 200                           | 400                                  | 200                               |
| Desiquam 222 <sup>a</sup> | 125                              | 125                               | 125                              | 125                           | 125                                  | 125                               |

NA = not amoebicidal at 1000  $\mu\text{g/ml}$ .

supplied with bacteria. Duplicate sets were run for drug dilution.

For testing drugs against axenically grown free-living amoebae; the method followed was the same as that of monoxenic culture, except that (1) media used for growing amoebae axenically<sup>14,15</sup> were used for drug evaluation. (2) sterile cover slips were used to cover the cavity to avoid outside contamination, (3) Petri-dish used for moist chamber was sealed with plastocin to maintain aseptic condition throughout the exposure period and, (4) slides inside moist chamber were placed in such a way so that observation could be taken under inverted microscope without opening the sealed petri-dishes (Fig. 1).

Drug dilutions were made by using sterile distilled water in the case of water soluble drugs and seitz filtered. In the case of insoluble compounds, the method of Das and Prasad<sup>13</sup> was followed. Antibiotics (Penicillin 500 units/ml and streptomycin 500  $\mu\text{g/ml}$ ) may be used in axenic medium to avoid outside contamination. Table I shows the test results of four known amoebicidal drugs, following this method.

The method described is novel and rapid, and can be used as a standard technique for routine screening of antiamoebic compounds *in vitro* where a good number of compounds are tested regularly everyday.

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#### EARLY AND MIDDLE PALAEOLITHIC TOOLS FROM RIVER TERRACES IN THE SAKETI AREA, MARKANDA VALLEY, HIMACHAL PRADESH

DURING the first joint venture undertaken in November-December, 1974, by the Geological Survey of India and the Deccan College Research Institute, Poona, Stone Age sites yielding Early and Middle Palaeolithic tools have been found in the Markanda valley. The sites occur on the river terraces close to Saketi village (Sirmur District, H.P.) where the G.S.I. is constructing Siwalik Fossil Park.

Five non-paired erosional terraces were observed at heights 50 m, 30 m, 18 m, 15 m and 5 m respectively above the present river bed. Of these the terrace at 18 m (Nagal terrace) is most extensive and the tools were found on this terrace as well as on the lower terraces. The terraces are composed of river gravel and silt and lie either on the Nahan or Tatrot, Pinjor or Boulder Conglomerate of the Siwalik formations of the area.

The tools are made on pebbles or pebble flakes of quartzite and comprise of choppers and scrapers. The choppers are mostly unifacial and only one or two show bifacial working. Handaxes and cleavers have not yet been found. Most interesting tool is a bifacially worked scraper on thin oval flake from pebble indicating advanced Acheulian characters. While the choppers clearly belong to Early Palaeolithic culture, the developed form of the bifacial scraper indicates existence of early Middle Palaeolithic culture in the area.

The Saketi region abounds in the Siwalik fossils belonging to Late Pliocene to Early Pleistocene periods. The Boulder Conglomerate is mostly non-fossiliferous. The river terraces which would be of post-Middle Pleistocene age have not so far yielded any fossils. It may be recalled that the Siwalik area has yielded fossils of some of the earliest primates, one of the rarest but by far the most important is *Ramapithecus* who is now generally accepted as being the precursor of mankind.

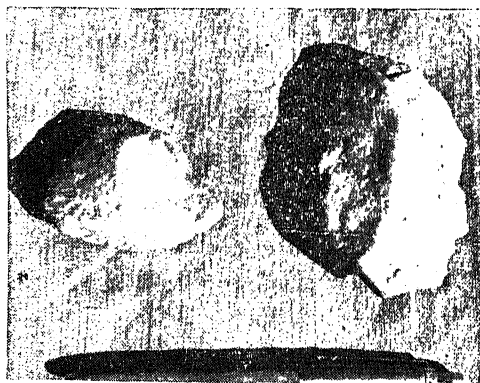


FIG. 1. Bifacially worked scraper on pebble flake and a simple flake.

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### EFFECT OF A COPPER INTRAVAS-CONTRACEPTIVE DEVICE ON BIOCHEMISTRY OF EPIDIDYMIS AND VAS DEFERENS IN MALE RATS

It has been observed earlier by Kar and Kamboj<sup>1,2</sup> that a surgical silk suture inserted in the vas deferens of rat produces sterility in about 70% of the animals. It has been reported that such a device does not occlude the lumen of the vas deferens and permits a free transport of spermatozoa which were normal and fertile<sup>3</sup>. The present study was undertaken to find out the effect of copper IVD on sperm transport.

Adult male rats (20) weighing 130–150 g were used in this study. Animals were divided into two groups of 10 each. In the first group, a piece of clean, soft, 100% pure copper wire (0.2 mm dia) was fitted bilaterally in the lumen of the vas according to the method of Chang and Tatum<sup>4</sup>. The operations were done under aseptic conditions and animals received post-operative antibiotics therapy for 4 days. The second group of rats were sham operated and served as controls. The animals were sacrificed 45 days after IVD insertion. Sperms were taken out from copper-containing region of vas and also from the device free area, placed in physiological saline.

Pieces of the vas deferens from different regions were fixed in Bouin's fluid and the sections were stained with Ehrlich's haematoxylin and eosin. Protein and urea content of the epididymis and vas deferens were determined by the techniques employed previously<sup>5,6</sup>.

Cu-IVD had no effect on the weight of testis and epididymis in rat (Table I). Microscopic examination of the spermatozoa from the copper IVD area exhibited cent per cent decapitation, whereas normal motile sperms were seen from the vas proximal to the epididymis, i.e., device free area. Sperms from cauda epididymis were normal and motile in about 50% of the animals. There was a significant fall in the protein concentration of the vas irrespective of presence or absence of device in copper IVD group (control vs copper device area or device free area,  $P < 0.01$ ). Likewise the urea content of the vas registered a significant rise after IVD insertion (control vs copper device

TABLE I

Effect of Cu-IVD on the protein and urea composition of the epididymis and vas deferens

|                         | Control rats   | Cu-IVD group   |               |
|-------------------------|----------------|----------------|---------------|
|                         |                | Cu-IVD side    | Free side     |
| Testis weight (mg)      | 1077 ± 20.70   |                | 1090.6 ± 18.4 |
| Epididymis weight (mg)  | 218 ± 9.15     |                | 182 ± 6.72    |
| Epididymis vas deferens | Protein (mg/g) | 206.16 ± 21.77 | 125.3 ± 12.15 |
|                         | Urea (mg/g)    | 0.85 ± 0.08    | 2.88 ± 0.07   |
| Epididymis              | Protein (mg/g) | 145.94 ± 7.27  | 105.0 ± 5.62  |
|                         | Urea (mg/g)    | 0.45 ± 0.07    | 1.80 ± 0.04   |

area or device free area,  $P < 0.01$ ). However, the level of urea was higher in the Cu-IVD area than that of device free area of vas (device free area vs copper device area,  $P < 0.01$ ). There was a significant rise in the urea content of epididymides in Cu-IVD group (control vs Cu-IVD group,  $P < 0.01$ ).

Sperm granuloma was noticed in 80% of the cases at the epididymal end at the point of insertion of the device and in a few cases tissue adhesions with the adjoining fat were also observed. As reported in a previous study by Setty *et al.*<sup>3</sup> using a nylon IVD for a short term, the copper did not alter the weights of epididymis and testis. It was further noted that the vasal lumen was only partially occluded by the copper wire and it allowed a free passage of spermatozoa. High content of urea recorded in the vas deferens and epididymis portion of the copper IVD group is a significant finding and indicates that copper IVD not only interferes at the vasal level but also at the epididymal level.

Studies on fertility performance showed that out of ten female rats, none could be mated by five copper-IVD inserted male rats probably due to the high percentage of sperm granuloma formation.

Technical assistance of Miss R. Mendiratta is acknowledged.

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#### A NEW LEAF SPOT DISEASE OF GROUNDNUT CAUSED BY *LEPTOSPHAERULINA TRIFOLII* (ROSTR.) PETRAK

A NEW type of leaf spot was observed on groundnut (*Arachis hypogaea*) in Agricultural Research Station, Aliyarnagar, Tamil Nadu. The spots were circular and brown in the early stages. At maturity they had grey centre and brown margin and measured 2–3 mm in diameter. A large number of minute black dots were observed in the centre of the lesion on the upper surface of the leaf (Fig. 1). The fungus *Leptosphaerulina trifolii*

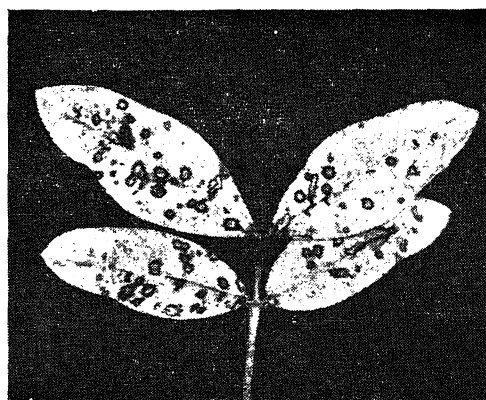


FIG. 1. Symptoms produced by *Leptosphaerulina trifolii* on groundnut leaves.

(Rostr.) Petrak was isolated into pure culture and its pathogenicity to groundnut proved. It was re-isolated from inoculated plants and was identical to the original isolate.

Sechet<sup>1</sup> proposed the name *Pleospora crassiasca* to the pathogen causing a similar disease in groundnut in Madagascar. Jackson and Bell<sup>2</sup> concluded that the pathogen should be named *Leptosphaerulina arachidicola* Yen, Chen. and Huang. They named the disease as leaf scorch and pepper spot. This species has been reported on groundnut from Formosa, U.S.A., Argentina and also Hyderabad<sup>3-6</sup>.

The present report is the first one of *L. trifolii* attacking groundnut. This species is reported to attack *Trifolium repens*, *T. pratense*<sup>7</sup>, wheat<sup>8</sup>, *Aponogeton crispus*<sup>9</sup>, *Passiflora leschenaultii*, *Marsilia quadrifoliata*<sup>10</sup> and *Cassia* spp.<sup>11</sup>. The symptoms produced by *L. arachidicola* in leaves include well defined marginal desiccation in nature<sup>1</sup> and fleck to scorch upon artificial inoculation<sup>2</sup>. But *L. trifolii* produced spots of 2-5 mm diameter dispersed on the entire lamina both in natural and artificial infections. The specimens have been deposited in the Herbarium of the Plant Pathology Department, Tamil Nadu Agricultural University, Coimbatore 641 003.

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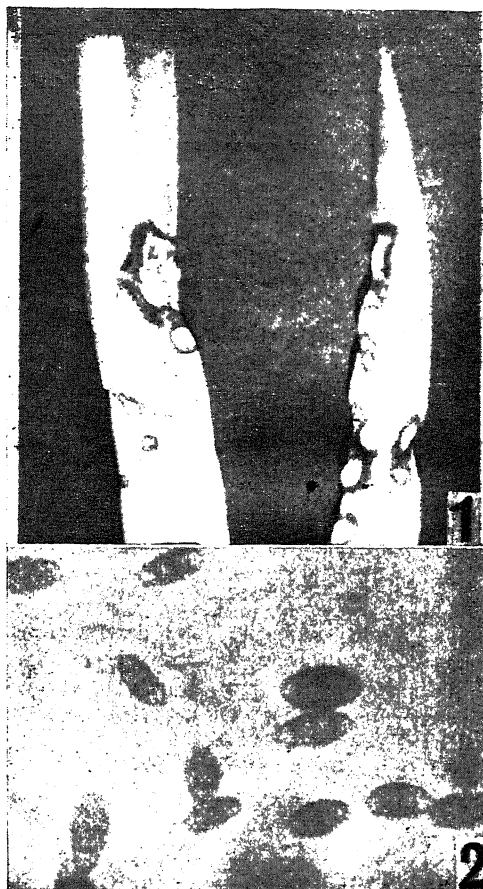
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## NOTES ON TWO CURVULARIA LEAF BLIGHTS ON ECONOMIC CROPS IN ASSAM

OCCURRENCES of two hitherto unrecorded *Curvularia* diseases in India both causing leaf blights, viz., *C. eragrostidis* (P. Henn.) J. A. Meyer<sup>1</sup> on pineapple (*Ananas sativus* Schult.), observed in August, 1974 and *C. andropogonis* (Zimm.) Boedijn<sup>2</sup> on lemon grass [*Cymbopogon citratus* (DC.) Stapf.], recorded in August, 1972, are reported in this communication.

**Leaf blight of pineapple.**—Symptom develops on leaves as elliptic, oblong or almost globose yellowish spots which increase in size (very small to over 5 cm long) changing to different shades of brown with straw-coloured centres and prominent brown margins. These spots become depressed and a

yellow halo persists at the beginning; a few spots may coalesce and ultimately the leaves die due to blighting (Fig. 1).



FIGS. 1-2. Fig. 1. Pineapple leaves (cv. Kew) showing typical symptom of blight due to *Curvularia eragrostidis*. Fig. 2. Conidia of the fungus on PDA.

Conidiophores with bulbous base arise in groups of 2 to 4 (inconspicuous stoma of a few cells usually present), 5-7 septate, (100-) 150-282 × 6.6-8.3 (-9.1) μ; conidia olivaceous brown to medium brown, 3 septate, end cells lighter, middle septum almost median, ellipsoidal, symmetrical with prominent scar, (18.3-) 21.6-24.9 (-26.6) × (9.1-) 10.8-11.6 (-13.3) μ (Fig. 2). Formation of conidia on PDA starts after 2 days (conidiophores develop from mycelium or pin-like stromatic masses) and occasionally minute black sclerotia develop at the edge (I.T.C.C., 1909).

Inoculation on the wounded leaves of 'Kew' and 'Queen' in saturated atmosphere produced the symptom after 2 to 3 days and on unwounded leaves, after 7 to 8 days.



*Leaf blight of lemon grass.*—Symptom develops as purplish linear lesions on the margins or tips of the leaves which gradually advance inwards blighting the tissues. An interesting observation was made that both the length and breadth of conidia were significantly reduced (at 1% level) on PDA-length, on leaf  $35.5-56.4 \mu$  and on PDA  $24.9-48.1 \mu$  (Student  $t = 7.15$ ); breadth, on leaf  $14.9-21.6 \mu$  and on PDA  $11.6-19.9 \mu$  (Student  $t = 3.79$ ).

Thanks are due to Shri U. S. Das for helping in statistical analysis and Shri M. C. Sharma for taking record in my absence.

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### CYTOTOXICITY OF SERUM AND CEREBRO-SPINAL FLUID FROM CASES OF ACUTE ENCEPHALOPATHY

DURING last five years we have done virological, bacteriological, serological and haematological studies in 156 cases of acute encephalopathy to establish their etiology. These cases have been occurring in children with high mortality in epidemic proportions. Their cerebrospinal fluid

(CSF) presented no abnormal finding. The only positive finding was the presence of one or more pathogenic bacteria in throat, stool and blood. On the basis of clinical manifestations and presence of pathogenic bacteria at one or the other site in the body (Table I), it was concluded that involvement of brain occurs through toxins produced by the bacteria in 40% of the cases<sup>1,2</sup>.

During attempts to isolate virus (if any) in monkey kidney tissue culture from CSF and serum samples, toxic granules were observed with some of the specimens, indicating presence of some toxic substance. We have observed that chick embryo tissue culture is highly sensitive to bacterial toxins<sup>3</sup>. Therefore, serum and CSF obtained from 36 patients were inoculated in primary chick embryo tissue cultures (CEC). For controls, cases who were not suffering from fever, encephalopathy or any infectious disease were also included. Sera from 20 and CSF from 12 of such control cases were investigated. The culture tubes were incubated at  $37^{\circ}\text{C}$  and watched for cytotoxicity at 30 minutes interval upto 4 hours. The grading of cytotoxic damage was done as reported earlier<sup>3</sup>. Cytotoxic damage to the cell sheet was observed with serum alone from 15 patients, serum and CSF both, in another five patients (Table II). Some of the specimens were cytotoxic upto a dilution of 1:16. The cytotoxic effect was not observed on subsequent passage in tissue culture. None of the sera or CSF obtained from control cases was found to be cytotoxic.

TABLE I  
Bacteriological findings (156 cases)

|           | Throat swab |     |      | Rectal swab |                        | Blood |     |      | CSF |
|-----------|-------------|-----|------|-------------|------------------------|-------|-----|------|-----|
|           | SA          | Pn  | Ps   | Salm        | <i>F. coll</i><br>0:26 | Sh    | SA  | Salm |     |
| Total No. | 29          | 7   | 17   | 3           | 2                      | 1     | 15  | 16   | ..  |
| %         | 18.3        | 4.5 | 10.9 | 1.9         | 1.2                    | 0.6   | 9.6 | 10.2 | ..  |

CSF=Cerebrospinal fluid, Pn=pneumococcus, Ps=*Pseudomonas, Pyocyaneus*, SA=*Staphylococcus aureus*, Salm=*Salmonella typhi* and *paratyphi*, Sh=*Shigella dysenteriae*.

TABLE II  
Cytotoxicity in chick embryo tissue culture

| Group                | Cytotoxicity |     |      |     |     |      |           |     |      |
|----------------------|--------------|-----|------|-----|-----|------|-----------|-----|------|
|                      | Serum        |     |      | CSF |     |      | Serum+CSF |     |      |
|                      | No.          | +ve | %+ve | No. | +ve | %+ve | No.       | +ve | %+ve |
| Acute encephalopathy | 36           | 15  | 41.4 | 36  | 0   | 0    | 36        | 5   | 13.8 |
| Control              | 20           | 0   | 0    | 12  | 0   | 0    | 12        | 0   | 0    |

Thus, demonstration of cytotoxic damage by the samples from 20 out of 36 patients (55.5%) shows presence of toxic substances in serum or CSF. This further supports our view that in some of the cases, brain involvement is due to toxins<sup>1,2</sup>. The nature of toxins is being investigated.

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#### OCCURRENCE OF *ACETES AUSTRALIS* *COLEFAX* AND *ACETES VULGARIS* HANSEN IN COASTAL WATERS OF INDIA

DURING a detailed study of the systematics of the genus *Acetes* H. Milne-Edwards, occurring in different parts of the West coast of India, a few specimens of *Acetes australis* Colefax and *Acetes vulgaris* Hansen<sup>2</sup> including both males and females were obtained. The present account, as far as the author is aware, records their occurrence for the first time in the Indian coastal waters.

##### *Acetes australis* Colefax :

Males : 2—17.1 mm and 16.3 mm in total length, collected off Cochin on 26-3-1973.

Females : 4—16.0 mm to 20.7 mm in total length, collected off Cochin on 26-3-1973.

Specimens were found in a plankton collection along with *A. erythraeus*<sup>3</sup> and *A. cochinesis*<sup>4</sup>. All the specimens were adults showing the specific characters of *A. australis* reported from the Australian waters by Colefax<sup>1</sup>, the similarities to which are listed below.

Lower antennular flagellum of male with 11 segments carrying a single clasping spine and accessory spines; segment anterior to the one carrying the clasping spine bearing 3 spines and projection; 4 spines on the segment opposing the tip of clasping spine endopodite of 2nd maxilla bearing short setae on its distal inner margin and a small conical projection with short bristles at its tip; exopodite of 1st maxilliped carrying bristles at its distal outer margin and short spiny projection on its inner margin; procurved spine on the sternum between the 1st pair of pleopods absent; blunt projection on the basis of 3rd pair of legs; coxae of 3rd pair of legs carrying tooth in males and

females; modified endopodite of 2nd pleopod of male carrying a lamella with 4 spines at its tip; petasma having pars externa, pars astringens and pars media; single falcate spine on capitulum and a few small spines at the tip; tip of processes ventralis pointed; 3rd thoracic sternite of female carrying 2 pairs of protuberances, one at the anterior margin and the other behind in contact with coxal expansion and a wide shallow groove running to the anterior margin of 4th sternite.

From the original description of the species some minor difference noticed in the present material are mentioned below.

The segments of the antennular peduncle in the proportion 21 : 7 : 14 instead of 22 : 7 : 16; lower antennular flagellum of female 22-segmented as against 24; 3rd leg unlike in the typical one reaching behind the antennal scale; abdomen 2.9 times the length of cephalothorax and segments in the proportion 9 : 8 : 10 : 11 : 18 : 17 and not 2.7 and 9 : 8 : 9 : 11 : 9 : 19 respectively.

##### *Acetes vulgaris* Hansen

Males : 3—15.3 mm to 18.9 mm in total length, from Goa coast collected on 24-1-1974.

Females : 7—16.8 mm to 17.6 mm in total length, from Goa coast collected on 24-1-1974.

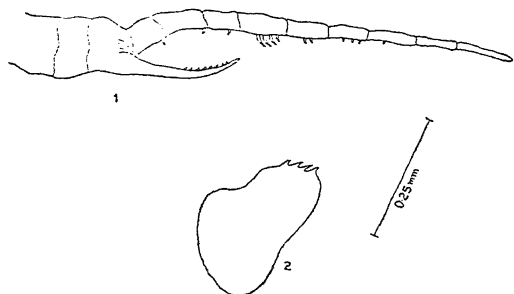
*A. vulgaris* has been first reported by Hansen<sup>2</sup> from Surabaya, Cheribon, Malakka and Kob Kobdat during the Siboga Expedition. The present specimens were found along with a closely allied species, *A. sibogae* Hansen recorded from Travancore coast by Nataraj<sup>5</sup>. *A. vulgaris* collected by the author resembles the typical species in the following features.

3rd segment of antennular peduncle of male almost equalling in length to that of female; 16-20 segmented antennular flagellum in female; single clasping spine on the lower antennular flagellum in male; 4-5 spines on the segment opposing the tip of clasping spine; procurved tooth on the sternum between the first pair of pleopods absent; basis of 3rd pair of legs without tooth; coxal tooth on the 3rd pair of legs both in males and females; well developed pars externa pars astringens and pars media of petasma; 2 large hooks and few minute spines on capitulum; a pair of rounded protuberances on the 3rd thoracic sternite of female and a deep transverse furrow.

Some minor differences, met with in the specimens examined from the Goa region but not shown by Hansen in his figures and description relating to this species, are listed below.

Lower antennular flagellum of male 12-segmented bearing small triangular teeth in the median shallow groove of the clasping spine (Fig. 1); modified

endopodite of 2nd pleopod of male carrying a lamella with 4 curved hooks (Fig. 2); pars externa of petasma having a thickening at its distal outer margin; distinct small hook distal to the 2 large hooks of capitulum absent.



FIGS. 1-2. *A. vulgaris* Hansen. Fig. 1. Lower antennular flagellum of male. Fig. 2. Lamella of modified endopodite of 2nd pleopod of male.

The presence of these two species in the Indian coastal waters indicate that they have a wider distribution in the Indo-Pacific region than has hitherto been known.

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#### GIANT CELLS IN THE TESTES OF SWISS ALBINO MICE AFTER TREATMENT WITH TRITIUM

OCCURRENCE of two types of giant cells have been reported in the seminiferous tubules of rodents<sup>1-5</sup>, monkey<sup>6</sup>, and man<sup>7</sup> after high doses of irradiation, both external and internal.

Giant cells have also been noticed in mice after irradiation with tritium. Tritium, a heavy isotope of hydrogen with a half life 12.4 years, an atomic weight of 3, exists in nature in minute quantities and is also produced in atomic power reactors and nuclear explosions. In a series of experiments, a single dose of tritium in the form of tritiated water (20  $\mu$  Ci/ml of body water) was injected intraperitoneally at different age groups of mice from 1 week to 8 weeks. All the animals were autopsied after 72 hours of injection. Testes

were fixed in Bouin's fluid. After routine procedure sections, stained with haematoxylin and eosin, were observed for giant cells.

Two types of giant cells are observed in the seminiferous tubules of 5 week old and 6 week old injected mice. In one type, there is a single nucleus with large body of cytoplasm, i.e., hypertypic (Fig. 1). In another type, there are two to many nuclei in a common pool of cytoplasm (Fig. 2). Hypertypic cells are 2-5 in number

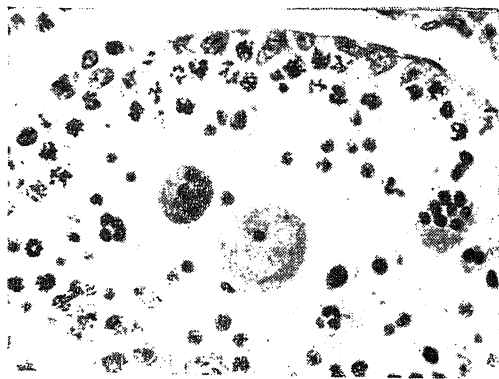


FIG. 1. Seminiferous tubule of 6 week old treated mice showing hypertypic and multinucleated giant cells,  $\times 300$ .

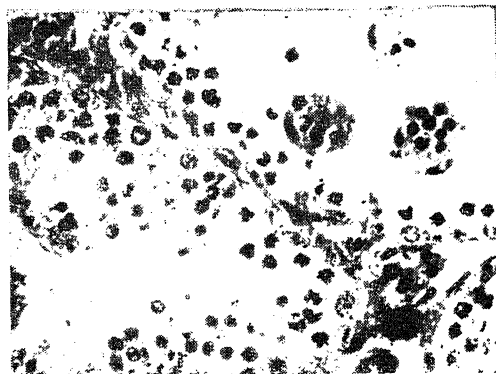


FIG. 2. Seminiferous tubule of 5 week old treated mice showing multinucleated giant cells,  $\times 300$ .

while multinucleated are many. Most of them are derived from young spermatids and rest of them being formed from the spermatocytes. These encountered cells in the mice testes are supposed to be either formed by multiple division in the absence of cleavage or by cell fusion. The multinucleated giant cells have also been reported in the gerbil testes after treatment with  $\text{Ca}^{45}$ ,  $\text{Co}^{60}$ , and  $\text{P}^{32}$  and are said to be formed by fatty degeneration of cell membranes<sup>8</sup>. Montgomery *et al.*<sup>9</sup> have found multinucleated giant cells in Chang liver cells

culture after X-ray irradiation formed by the fusion of plasma membrane. There is also a suggestion that the multinucleated giant cells are formed when macrophages swallow young spermatids<sup>3</sup>. But this view needs further studies.

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# **OCCURRENCE OF STREPTOCEPHALUS**

**SPINIFER GURNEY, 1906 (CRUSTACEA:**

**BRANCHIOPODA) FROM ANDHRA PRADESH**

*Streptocephalus spinifer* was described by Gurney<sup>1</sup> based on 3 females and 3 males collected by Mr. Green from Ceylon. Later Daday<sup>2</sup> redescribed the species on the basis of re-examination of the above material. The present report extends the distribution of the species to the Indian subcontinent.

While investigating the Branchiopod fauna of Guntur District, *S. spinifer* was collected from a temporary pool near Akaveedu in Prakasam District in Andhra Pradesh. A total of 28 specimens were collected on 12th and 20th August 1973 ; of these, three were females, the rest being males. All the specimens were preserved in 10% formalin.

Specimens slightly smaller than those reported from Ceylon. Males more slender, smaller than the common anostracan *S. dichotomus* Baird. Head

small, rounded, with a frontal process and a spiniform rostrum. The spiny armature of the abdomen differ considerably from the previous descriptions. Spines are present on the dorsal and lateral sides (Fig. 1) while in one specimen a rudimentary spine

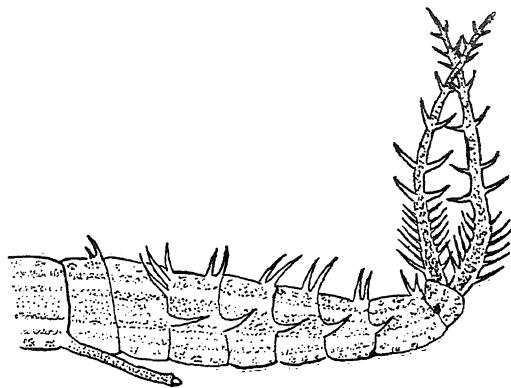


FIG. 1. *Streptocephalus spinifer*, posterior part of male.

is observed on the ventral side of the third abdominal segment. Critical examination of the abdominal segments of all specimens reveal the following spinal formula :

I 0, II 0-2, III 0-6, IV 0-4, V 2-4, VI 2-4, VII 2-4, VIII 0-4, IX 0.

Cercopods long bearing proximal small stiff setae and distal long fleshy spines. The number of setae and spines differ considerably in different individuals. In one specimen fleshy spines were totally absent.

Females more robust with narrow cylindrical ovisac extended upto 6 or 7 abdominal segment. The present specimens also differ from those described earlier by having ivory white colour with brilliant scarlet ovisac and cercopods.

The males of *S. spinifer* are distinguished by the presence of a curved spiniform rostrum and spines on the dorsal and lateral sides of the abdominal segments, and the smooth penis.

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# A NOTE ON PRESERVATIVES FOR RETAINING THE COLOUR OF SEAWEEDS

DIFFERENT algologists have suggested different liquid preparations for preserving algae for taxonomic purposes. West *et al.*<sup>1</sup> prefer 2.4% formalin as preservative. According to Smith<sup>2</sup> the simplest preservatives are 2.4% formalin or a mixture of formalin, acetic acid, and alcohol. Prescott<sup>3</sup> recommends formalin aceto-alcohol and Transeau's solution with glycerine as preservatives. Edwards<sup>4</sup> suggests, seawater mixed with commercial 40% formalin to obtain approximately a 3 to 5% solution, together with a little borax to act as buffer, comprises a good general purpose liquid preservative. Kamat<sup>5</sup> conducted some experiments for preserving the natural colour of freshwater algae

akinetes, *Volvox* sp., *Cladophora* sp., and also the *Euglena* spp., but it gives an unnatural colour to other chlorophyceae. The loss of colour of the algae may be retarded for few weeks if they are placed in dark place.

But none of the above preservatives was found successful to retain the colour of algae for longer periods and hence it was difficult to classify them into divisions like chlorophyceae, phaeophyceae and rhodophyceae, at first sight. Therefore, the work, to preserve the seaweeds with their natural colour for longer periods, was undertaken in 1972.

The solutions which were found most satisfactory as preservatives for the seaweeds are given in Table I.

TABLE I

| Preservative | Composition          |         | Seaweeds                               |
|--------------|----------------------|---------|--|
| 1            | Glycerine            | 60 ml   | 1. <i>Ulva</i> spp.                    |
|              | Formalin             | 60 ml   | 2. <i>Caulerpa</i> spp.                |
|              | Rectified spirit     | 6 ml    | 3. <i>Briopsis</i> spp.                |
|              | Copper sulphate      | 300 mg  | 4. <i>Halimeda</i> spp.                |
|              | Potassium dichromate | 15 mg   | 5. <i>Spongomorpha indica</i>          |
|              | Distilled water      | 2880 ml | 6. <i>Dictyosphaeria cavernosa</i>     |
|              |                      |         | 7. <i>Udotea indica</i>                |
| 2            |                      |         | 8. <i>Avrainvillea nigricans</i>       |
|              |                      |         | 9. <i>Valonia utricularis</i>          |
|              |                      |         | 10. <i>Chamaedoris auriculata</i>      |
|              |                      |         | 11. <i>Turbinaria ornata</i>           |
|              |                      |         | 12. <i>Hormophysa triquetra</i>        |
|              |                      |         | 13. <i>Hydroclathrus clathratus</i>    |
|              |                      |         | 14. <i>Colpomenia sinuosa</i>          |
|              |                      |         | 15. <i>Myriogloea sciurus</i>          |
|              | Glycerine            | 60 ml   | For all chlorophyceae and phaeophyceae |
|              | Formalin             | 60 ml   |  |
|              | Rectified spirit     | 6 ml    |  |
|              | Copper sulphate      | 200 mg  |  |
|              | Potassium dichromate | 15 mg   |  |
|              | Seawater             | 1900 ml |  |
|              | Distilled water      | 1000 ml |  |

in nine different preservatives and suggested that formalin aceto-alcohol was slightly better for most of the chlorophyceae and particularly desmids. He also suggested that Keefe's solution (50% alcohol 90 cc, formalin 5 cc, glycerine 2.5 cc, copper chloride 10 gm, uranium nitrate 1.5 gm) was good for preserving the green colour of some of the chlorophyceae like *Pilophora* sp., particularly the

Algae which were preserved in the above preservatives retain the colour still and they have been kept in the museum of the Marine Biological Research Station, Okba. They look attractive and freshly collected.

The red algae could not be preserved with colour except on herbarium sheets. Attempts have also been made to preserve the red algae with colour.

Author is grateful to the higher authorities of Fisheries Department, Government of Gujarat, for facilities provided.

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#### X-RAY INDUCED CHANGES IN THE LEVELS OF GLUCOSE, FRUCTOSE AND GLYCOGEN IN *PERIPLANETA AMERICANA*

X-RAYS have been successfully employed for the eradication of insects by inducing sterility into these organisms<sup>1</sup> and their effect on the biological functions of both the sexes have already been reported by earlier workers<sup>2</sup>. However, the effect of X-rays on glycogen, the reserve substance in insects, and sugars, which serve as the readily available source of energy have not yet been reported.

To study this aspect, adult cockroaches of either sex were subjected to whole body exposure of x-rays, at doses ranging from 1,200 to 8,400 rads. Following irradiation, glucose, fructose and glycogen were quantitatively estimated both in the midgut and hepatic caeca. The biochemical method adopted was that of Dubois, *et al.* (1956)<sup>3</sup> and the readings were tabulated as percentage decrease in the quantity (P.D.) of glucose, fructose and glycogen, from the control values. The unirradiated cockroaches served as controls.

**Results and Discussion.**—The observations clearly indicate that the quantity of glucose, fructose and glycogen both in the midgut and hepatic caeca were adversely affected following x-irradiation. The extent of decrease in quantity was dose dependent, *i.e.*, higher the dose applied, greater the decrease in quantity of the reserve substance glycogen, and the sugars glucose and fructose.

However an interesting feature is that P.D. of these metabolites in both the tissues studied was greater in the males than in the female insects, through all doses of exposure (Table I). In the males after an exposure dose of 8,400 rads the P.D. of glucose, fructose and glycogen was around ninety-five but in the females at the same dose it was only around sixty. This implies that the male cockroaches which are more sensitive to x-rays, suffer from a greater stress due to irradiation as compared

TABLE I

*Effect of X-rays on the levels of glucose, fructose and glycogen in cockroaches*  
(% decrease)

| Dose in rads        | Male cockroaches |      |      |               |      |      | Female cockroaches |      |      |               |      |      |
|---------------------|------------------|------|------|---------------|------|------|--------------------|------|------|---------------|------|------|
|                     | Midgut           |      |      | Hepatic caeca |      |      | Midgut             |      |      | Hepatic caeca |      |      |
|                     | Glu              | Fru  | Gly  | Glu           | Fru  | Gly  | Glu                | Fru  | Gly  | Glu           | Fru  | Gly  |
| Control* ( $\mu$ g) | (10)             | (15) | (30) | (9)           | (15) | (29) | (8)                | (12) | (24) | (8)           | (15) | (32) |
| 1200,               | 30               | 15   | 7    | 22            | 20   | 10   | 25                 | 8    | 4    | 13            | 7    | 6    |
| 2,400               | 40               | 33   | 27   | 58            | 50   | 50   | 38                 | 25   | 17   | 38            | 33   | 38   |
| 3,600               | 65               | 60   | 57   | 61            | 57   | 60   | 41                 | 29   | 25   | 41            | 40   | 43   |
| 4,800               | 84               | 80   | 80   | 82            | 80   | 77   | 45                 | 34   | 29   | 42            | 45   | 45   |
| 6,000               | 89               | 87   | 95   | 85            | 83   | 79   | 53                 | 45   | 42   | 53            | 55   | 54   |
| 7,200               | 96               | 95   | 98   | 95            | 93   | 88   | 56                 | 48   | 45   | 54            | 57   | 56   |
| 8,400               | 97               | 97   | 99   | 96            | 97   | 93   | 58                 | 50   | 46   | 56            | 58   | 58   |

\* Control values are expressed as  $\mu$ gs of glucose, fructose or glycogen/ml homogenate. The values for all the other doses indicate the percentage decrease in quantity/ml homogenate, of the control values.

to the females and hence utilise more sugar the readily available source of energy and glycogen the reserve substance<sup>4</sup>.

But the direct effect of x-rays may also be responsible for the drastic reduction in the quantity of glycogen, glucose and fructose, by mainchain scission of glycogen<sup>5</sup> and by converting the two sugars glucose and fructose to their corresponding uronic acids<sup>6</sup>. Therefore the decrease in quantity of glycogen, glucose and fructose both in the midgut and hepatic caused of cockroaches could be attributed to the stress caused due to x-irradiation of insects and to the direct effect of x-rays on the polysaccharide and the sugars.

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### ADAPTIVE SIGNIFICANCE OF HAEMOGLOBIN VARIANTS IN THE DESERT SHEEP OF RAJASTHAN

PRELIMINARY studies on Indian sheep (Magra) indicate that Hb confers some adaptive advantage<sup>8,9</sup>. The results of an earlier investigation conducted by

Singh *et al.*<sup>3</sup> revealed an excess of B lambs from B and AB matings and lead to undertake this investigation to further elucidate the adaptive significance of haemoglobin variants in the desert sheep of Rajasthan, as evinced by (i) frequency distribution of Hb-types, (ii) body weight of ewes and birth and weaning weights of their lambs and (iii) lamb production. A total of 832 sheep of different indigenous breeds (Magra, Chokla, Marwari, Jaisalmeri and Malpura) maintained at different farms of the state were selected for their Hb-type by paper electrophoresis using discontinuous system of buffers.

The gene frequency of Hb-B in all the breeds was very high-ranging from 0.663 in Chokla to 0.830 in Malpura sheep and are comparable to the previous reports<sup>4-7</sup>. Ewes with different Hb-types had insignificant differences in body weight except in Jaisalmeri sheep and the superiority of any particular Hb-types was not persistent in all the breeds; ewes having Hb-B had small persistent superiority in producing and weaning heavier lambs. Although AA individuals were rare, the birth rate with ewes with Hb-types, AA was lower than that of type AB and BB in all the flocks (Table I). However, the heterozygotes (AB) in Chokla and Magra sheep were a little superior to either of the homozygotes. Percentage of lambs surviving to weaning from ewes, having different Hb-types showed a little difference, though the lambs from AA individual had meagre chance of survival (Table I). Thus a little better reproductive performance appears to be associated with Hb-B; though available evidence does not suggest great bearing of Hb-types on lamb production.

The sheep breeds of Rajasthan are similar to the sheep breeds of Israel<sup>5</sup>, African Middle east<sup>1</sup> and

TABLE I  
Effect of Haemoglobin type of ewe on lamb production

|                            | Chokla (Avikanagar) |        |        | Malpura |        |       | Magra |        |        |
|----------------------------|---------------------|--------|--------|---------|--------|-------|-------|--------|--------|
|                            | A                   | AB     | B      | A       | AB     | B     | A     | AB     | B      |
| No. of ewes                | 10                  | 38     | 53     | 1       | 30     | 63    | 17    | 94     | 1000   |
| No. of lambs               | 31                  | 143    | 166    | 2       | 39     | 91    | 19    | 105    | 100    |
| expected                   | 33.66               | 127.92 | 178.41 | 1.40    | 42.13  | 88.46 | 18.05 | 99.83  | 106.20 |
| dropped { $\chi^2$ values  |                     | 2.815  |        |         | 2.557  |       |       | 0.739  |        |
| No. of lambs               | 23                  | 122    | 136    | 2       | 32     | 76    | 19    | 97     | 86     |
| weaned { expected          | 27.82               | 105.72 | 147.45 | 1.17    | 35.10  | 73.72 | 16.27 | 89.96  | 95.70  |
| weaned { $\chi^2$ values   |                     | 4.232  |        |         |        | 0.934 |       | 1.992  |        |
| Survival lambs             | 0.72                | 0.853  | 0.819  | 1.000   | 0.821  | 0.835 | 1.000 | .920   | .860   |
| of upto weaning { $\chi^*$ | 2.2329              |        |        |         | 0.6670 |       |       | 4.4444 |        |

\* values.

Sudan desert<sup>2</sup> and are predominantly H-b type. There is little superiority of Hb-B in overall lamb production, their survival rate, and their body weight at birth weaning. Since these measures are indicative of mothers' as well lambs' propensities, it appears that probably the Hb-A has lower adaptive significance, indicating the superiority of Hb-B in Rajasthan environment.

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#### AMINO ACID AND NUCLEIC ACID COMPOSITION OF THE POLYHEDRAL INCLUSION BODIES OF THE NUCLEAR POLYHEDROSIS VIRUS OF *SPODOPTERA LITURA* (FABRICIUS)

THE nuclear polyhedrosis viruses (NPV) of insects are among the well studied insect pathogens. Proteins and nucleic acids are the major constituents of polyhedral inclusion bodies (PIB) with virus particles. Amino acid and nucleic acid composition of the inclusion bodies of NPV of *S. litura* are reported herein.

Inclusion bodies were obtained from the infected last instar larvae of the tobacco caterpillar, *Spodoptera litura*. PIB were purified differential and density-gradient centrifugation<sup>1</sup>. Purity of the PIB was ascertained by electronmicroscopy. Amino acids of PIB after HCl hydrolysis were determined

using Technicon-sequential multisample (TSM) amino acid autoanalyzer.

The nucleic acids were extracted from three replicates of PIB (20 mg) in 0.5 N HClO<sub>4</sub> by treating for 1 hour on a water bath at 70° C as described by Faust<sup>2</sup>, and DNA and RNA contents were determined using diphenylamine and orcinol reagents respectively, as outlined by Schneider<sup>3</sup>. Exherring sperm highly purified DNA (Koch-Light Laboratories, England) and yeast RNA (BDH, England) were used as standards.

Amino acid composition of the PIB of *S. litura* is presented in Table I. Ratio of basic to acidic amino acids was 1.04. In general, the amino acid pattern of *S. litura* PIB was strikingly similar to those previously reported from other NPV in insects<sup>4</sup>.

TABLE I

*Amino acid composition of polyhedral inclusion bodies of NPV of S. litura\**

| Amino acid    | %    | Amino acid    | %    |
|---------------|------|---------------|------|
| Lysine        | 8.3  | Histidine     | 4.4  |
| Arginine      | 8.6  | Aspartic acid | 10.0 |
| Threonine     | 2.7  | Serine        | 3.6  |
| Glutamic acid | 10.2 | Proline       | 4.3  |
| Glycine       | 3.0  | Alanine       | 4.2  |
| Cystine       | 1.8  | Valine        | 5.2  |
| Methionine    | 3.2  | Isoleucine    | 4.1  |
| Leucine       | 7.6  | Tyrosine      | 10.9 |
| Phenylalanine | 6.3  |               |      |

\* Tryptophan content was not estimated; Correction for nucleic acid glycine was not made.

Hayashi and Durzan<sup>2</sup> suggested that the ratio of basic and acidic amino acid residues can help to characterize the viral proteins. On this basis, ratios calculated for combined inclusion body and virus proteins reported earlier and in the present study are: 1.0, 0.79 and 1.04 respectively for PIB of *Peridroma saucia*<sup>6</sup>, *Heliothis zea*<sup>7</sup> and *S. litura*. These results would indicate that PIB of *H. zea* are more acidic than the other two viruses.

The PIB of *S. litura* contained DNA and RNA in amounts of 14.0 ± 1.41 and 6.26 ± 0.74 µg/mg of PIB, respectively. Qualitatively these findings agree with those reported for other polyhedral viruses<sup>1</sup>. The amounts of DNA and RNA obtained are lower (approximately 35 and 50% respectively,



for DNA and RNA) than those reported for PIB from *Spodoptera exigua*<sup>2</sup>, a closely related species. The ratio of DNA : RNA for PIB of *S. litura* NPV was 2.2 which compared well with 1.5 and higher for all NPV reported previously except the silk worm NPV and is close to that of 2.4 for PIB of zebra caterpillar, *Ceramica picta*<sup>8</sup>.

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#### CORRELATION STUDIES BETWEEN PROTEIN PERCENTAGE AND PELSSENKE VALUE IN TWO WHEAT CROSSES

PROTEIN content and Pelschenke value are very important characters in wheat which reflect actually the bread making quality. The bakers depend upon the quality of the gluten which is measured by Pelschenke value. The present study reveals the scope of improving both the characters simultaneously by selection.

Crosses were made between  $C_{273} \times S_{227}$  and  $C_{273} \times S_{308}$  and after developing the material upto F<sub>4</sub> generation, experiments were laid out in a randomized block design in two replications. Individual plants of P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub> and F<sub>2</sub> were threshed

separately whereas F<sub>3</sub> and F<sub>4</sub> plants were threshed separately but bulked later on. Flour protein content was estimated by using Dye Binding Capacity Method using Udy Analyzer. Pelschenke value was determined following the method given by Austin *et al.* (1967). These estimates were made in the Quality Testing Laboratory at Indian Agricultural Research Institute, New Delhi. The data on individual plant was tabulated and phenotypic correlation coefficient was worked out (Table I).

TABLE I

*Phenotypic correlation between flour protein percentage and Pelschenke value*

| Generation            | 'r' value<br>( $C_{273} \times S_{227}$ ) | 'r' value<br>( $C_{273} \times S_{308}$ ) |
|-----------------------|---|---|
| F <sub>2</sub>        | +0.2444                                   | +0.3880**                                 |
| F <sub>3</sub> (1968) | +0.0414                                   | +0.0619                                   |
| F <sub>3</sub> (1969) | +0.1075                                   | +0.1107                                   |
| F <sub>4</sub>        | +0.1065                                   | +0.0360                                   |

Positive correlation coefficient was observed between flour protein content and Pelschenke value which was very small in magnitude. In case of F<sub>2</sub> only the coefficient value reached significance level with *r* value being low. This significant correlation could have been used as a tool in making selection simultaneously for both characters had the magnitude been large. The coefficients were too small to be of value for prediction purposes. Significant positive correlation between protein content and Pelschenke value has been found by several workers (Majsterenko *et al.*, 1964; Sarazin, 1965; Cutler *et al.*, 1933; Winter *et al.*, 1934).

In the present study it has been found that some high protein lines were low in Pelschenke value but most of the high Pelschenke lines were also high in protein content. This information favours the conclusion that certain protein genes have pleiotropic effect on Pelschenke value but overrules the possibility of strong linkage between genes responsible for protein content and Pelschenke value. The data also suggest that the selection for high Pelschenke value might also result in the selection for high protein content. Therefore, Pelschenke Test can be used as an efficient tool in the simultaneous improvement of both the characters since the Test is quicker and inexpensive.

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#### THERMOCONTROL OF SEED GERMINATION IN *SISYMBRIUM IRIO*

THERE have been numerous studies on seed germination<sup>1-3</sup> but the mechanism and factors, regulating it remain unsolved problems. The seed is considered 'an adaptive mechanism to facilitate suspending growth and interrupting the homeostatic continuum in the life cycle to overcome unfavourable conditions of growth'. The present communication reports the germination behaviour of the seeds of *Sisymbrium irio* (Cruciferae) which is a common herbaceous winter weed.

The seeds germinate during October-November and flowering starts in about two months. Seeds ripen during March-April. The seeds for the present study were collected from the plants in the University Campus, Jaipur, during March 1974. Usual methods were employed to study germination behaviour and two to three replicates were taken for each treatment. The results are based on averages of observations on 150 to 200 seeds in each treatment.

The freshly collected seeds were placed for germination at several temperatures between 15 and 40° C. While no germination occurred at temperatures other than 15-16° C, cent per cent synchronous germination was observed on the fifth day at 15-16° C. Light was not necessary for germination.

Later, the seeds were subjected to low temperature (15-16° C) for varying periods and then placed for germination at 25 and 30° C. The results summarised in Table I show that the seeds require a minimum of 8 hours treatment at 15° C

to induce germination. Slightly longer cold treatment (14 hr) yields 100% germination at 25° C but much longer treatment (36 hr) is required for the same effect at 30° C.

TABLE I  
Germination of *S. irio* seeds treated at 15° C

| Duration at 15° C (hr) | 25° C | % germination at 30° C |
|------------------------|-------|------------------------|
| Upto                   |       |                        |
| 6                      | 00    | 00                     |
| 8                      | 21    | 4                      |
| 10                     | 31    | 8                      |
| 12                     | 66    | 15                     |
| 14                     | 100   | 35                     |
| 16                     | 100   | 50                     |
| 18                     | 100   | 56                     |
| 20                     | 100   | 87                     |
| 22                     | 100   | 81                     |
| 24                     | 100   | 85                     |
| 36                     | 100   | 100                    |
| 48                     | 100   | 100                    |

The seeds were next subjected to low temperature for 2 to 12 hours in 24-hr cycle and kept either at 25 or 30° C for the remaining period. The results (Table II) indicate clearly that an increase in the duration of low temperature treatment in each cycle reduces the number of cycles required for maximum germination and also that it yields higher germination.

TABLE II  
Germination of *S. irio* seeds after various cycles of low temperature treatment

| Duration at 15° C in each 24-hr cycle | Maximum germination at 25° C | Maximum germination at 30° C |
|---------------------------------------|------------------------------|------------------------------|
| 2 hr                                  | 00 (7)*                      | 00 (7)*                      |
| 4 hr                                  | 18 (7)                       | 00 (7)                       |
| 6 hr                                  | 28 (7)                       | 18 (7)                       |
| 8 hr                                  | 100 (7)                      | 31 (7)                       |
| 10 hr                                 | 100 (3)                      | 76 (7)                       |
| 12 hr                                 | 100 (2)                      | 100 (3)                      |

\* Figures in parentheses denote the number of cycles required to obtain given germination.

It is also noteworthy that more germination is obtained at 25° C than that at 30° C. It appears

from the preliminary results obtained here that low temperature is required to activate the embryo but the reversion to a higher temperature within 8 hours suppresses or completely inhibits this activity. It is also indicated that low temperature for about 14 hours is required to initiate the growth activity and that further growth of the embryo is favoured by higher temperatures.

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#### CHEMICAL CONTROL OF DECAY OF FRUIT OF *VITIS VINIFERA* CAUSED BY *ASPERGILLUS* *NIGER* AND *PENICILLIUM* SP.

In the local markets, *Aspergillus niger* and *Penicillium* sp. were found to be causing severe deterioration of grape berries. In both the cases the infection started from skin cracks or an injury to the fruit. The healthy fruits in the bunches soon became infected when they came in contact with the diseased ones. Within 5 days the berries were completely destroyed by the black moldy growth of *A. niger*, whereas only 20% decay due to *Penicillium* sp. occurred in the individual fruit. A dirty olive green growth of *Penicillium* sp. appeared on the infected regions.

The efficacy of zinc ethylene bisdithiocarbamate (Dithane Z-78), zinc ion and manganese ethylene bisdithiocarbamate (Dithane M-45), dinitro (1-methyl heptyl) phenyl crotonate (Karathane), 4 *n*-butyl-1, 2, 4-triazole (RH-124), N-(Trichloromethyl) thio-4-cyclohexene-1, 2-di-carboximide (Captan) and O-Diisopropyl-5-benzyl thiophosphate (Kitazin) was tested at varying concentrations (500, 1000 and 2000 ppm).

Just ripe healthy fruits of grapes were inoculated with *A. niger* or *Penicillium* sp. by pin prick method. They were incubated for 8 hours before they were treated with the respective fungicide. For determin-

ing the effect of chemicals the inoculated fruits were immersed for 10 minutes in the respective solutions and for each treatment there were 10 replicates. For control, uninoculated fruits treated similarly were run simultaneously. The treated as well as the check fruits were placed in separate polyethylene bags and incubated for 4 days at  $25 \pm 2^\circ \text{C}$ .

Out of the fungicides tried, Dithane Z-78, Dithane M-45, RH-124 and Kitazin failed to control the rot caused by *A. niger*. Karathane at 2000 ppm reduced the losses upto 20% whereas the other two concentrations of this fungicide failed to control the *Aspergillus* rot. Captan reduced the loss upto 70% when used at 2000 ppm, even its lower concentrations were effective in reducing the loss due to decay. Higher concentrations (1000 and 2000 ppm) of Dithane Z-78, Dithane M-45 and RH-124 reduced the losses caused by *Penicillium* sp. but Karathane and Captan proved more effective in comparison to the other fungicides as at concentration of 2000 ppm they controlled the rot upto 70% and 85% respectively. Kitazin at 2000 ppm reduced the loss caused by *Penicillium* sp. upto only 15% but the same fungicide in concentrations of 500 and 1000 ppm proved ineffective. Chand *et al.*<sup>1</sup> found Karathane as a preinoculation spray to control the infection of *Alternaria citri* causing rot of citrus fruits. Gangopadhyay and Kapoor<sup>2</sup> reported that Captan reduced the losses to about 50% when its 1% suspension was sprayed on the fruits of *Cucurbita pepo* infected with *Alternaria cucumerina*.

Except very slight smell of Karathane observed in the flavour of juice extracted from fruits treated with this fungicide, no phytotoxic effects on the fruit of grapes were produced by any of the fungicides used in the present investigation. From the present findings Captan may be recommended for reducing the decay of grapes caused by *A. niger* and *Penicillium* sp.

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# CUBA 19, A HIGHLY HETEROTIC VARIETY OF MAIZE AND ITS POSSIBLE UTILIZATION IN VARIETAL HYBRIDS

IN recent years, the nature of gene action<sup>1</sup> and the results of selection experiments<sup>2</sup> have adequately demonstrated that varietal hybridization accompanied by population improvement in maize can be as effective as conventional hybrid approach. This basic understanding accompanied by the availability of wide spectrum of variability revived interest in intra- and inter-population improvement programmes. In the Indian maize programme, elite exotic materials have been systematically evaluated along with indigenous collections and the promising ones have been used for breeding composites. Such populations are expected to give high and stable performance under varied experiments.

During the present investigation, a diallel set of 20 elite yellow maize composites/synthetics/open-pollinated varieties was grown in the Indo-Gangetic plains and in the submontaneous *tarai* belt during *kharij*, 1970 and 1971<sup>3</sup>. Out of the varieties studied, Cuba 19 showed most promising heterotic responses (Table I).

Positive heterosis over the mid-parent was recorded in all the 19 crosses involving Cuba 19 in each of the four environments (except Antigua 3 D × Cuba 19). Superiority of F<sub>1</sub> hybrids over the better parent was also observed in all possible 76 instances (19 hybrids in four environments) except in ten, half of these ten were marginal cases,

For grain yield averaged over the environments, the heterosis (over the mid-parent) ranged from 6 to 40%. Except for Antigua 3 D × Cuba 19, all hybrids outyielded the better parent. It is of particular importance since heterosis in first five hybrids represented gain over the commercially released composites (Amber, Jawahar, Kisan, Vijay and Vikram). For a critical evaluation of commercial feasibility, the heterosis over the best composite, Jawahar, in present study, was also calculated. It was interesting to note that most of the hybrids were superior to the best composite, the increase being 3 to 18%. The Kisan × Cuba 19 hybrid showed consistent performance in all the environments, with 18% bonus, on the average, which was the highest.

TABLE I

*Per cent heterotic responses of hybrids involving Cuba 19 as recurrent parent*

| Hybrid Cuba 19x   | Mid parent |        | Better parent |          | Heterosis over Jawahar |
|-------------------|------------|--------|---------------|----------|------------------------|
|                   | Heterosis  | Range‡ | Heterosis     | Range‡   |                        |
| Amber             | 20         | 6-34   | 13            | 4-22     | 3                      |
| Jawahar           | 20         | 8-40   | 9             | *-2-27   | 9                      |
| Kisan             | 40         | 34-46  | 35            | 27-38    | 18                     |
| Vijay             | 19         | 4-44   | 10            | *-8-30   | 7                      |
| Vikram            | 22         | 4-34   | 17            | *-16-32  | 5                      |
| C 2               | 33         | 15-100 | 32            | 5-98     | 9                      |
| J 236             | 15         | 6-34   | 5             | 2-8      | 3                      |
| Prolific          | 28         | 18-52  | 22            | 3-45     | 10                     |
| A 22              | 19         | 8-41   | 8             | ** -3-29 | 8                      |
| A 23              | 33         | 16-55  | 31            | 2-52     | 11                     |
| E 13              | 21         | 6-39   | 13            | 4-24     | 6                      |
| Antigua Gr. I     | 28         | 16-43  | 25            | 5-28     | 6                      |
| Antigua 2D        | 26         | 0-48   | 21            | *-1-45   | 6                      |
| Antigua 3D        | 6          | *-9-12 | -4            | ** -26-2 | -2                     |
| Caribbean Flint   | 12         | 2-32   | 4             | *-10-25  | -1                     |
| Puerto Rico Gr. I | 18         | 11-32  | 10            | 2-18     | 5                      |
| St. Groix 4D      | 25         | 2-51   | 15            | *-11-31  | 11                     |
| Yellow Tuxpeno    | 28         | 15-65  | 25            | 11-64    | 7                      |
| Francisco Flint   | 23         | 10-32  | 19            | 8-29     | 4                      |

‡ Range over four environments.

\*, \*\* indicate that hybrid had negative heterosis in one and two environments, respectively.

*Implication*

Effective utilization of heterosis depends primarily on the nature of gene action involved. Crow<sup>4</sup> computed the expected heterosis based on dominance hypothesis and observed that with complete dominance of all favourable genes, an increase of 5 to 6% over mid-parent might be obtained. In the present study the increase over mid-parents ranged from 6 to 40%. The high heterotic response might be due to non-additive mode of inheritance.

If large proportion of genetic variability was accounted for by the additive and additive  $\times$  additive epistasis—the fixable component, the heterotic hybrids are likely to show low inbreeding depression and their advance generations hold promise as a composite. One such composite is 'Sona', an advance generation of the varietal hybrid  $J_1 \times$  Cuba 11 J. The best hybrids, in the present study, may be advanced and evaluated for this possibility. Another alternative is to develop a composite of these hybrids. Better performance of Cuba 19 in all the hybrids indicates high general combining ability and additive or/and additive  $\times$  additive gene action. Hence, Cuba 19 may be used as a parent to develop composites.

If dominance or/and epistasis interactions involving dominance (dominance  $\times$  dominance and additive  $\times$  dominance) are involved in the expression of heterosis, the  $F_1$  varietal hybrids should be directly utilised for commercial cultivation. Kisan  $\times$  Cuba 19 seems to be a promising hybrid. In developing countries the varietal hybrids have better commercial feasibility than conventional hybrids as their seed production is relatively simpler. Varietal hybrids have added advantage since these are amenable to further improvement.

The heterosis also depends upon the genetic diversity among parents. Assuming the varieties to be at equilibrium between mutation and selection and if gene frequencies affecting yield were similar in various populations, the extent of heterosis obtained indicates that Cuba 19 differed from other maize populations under study with respect to genetic constitution. Cuba 19 was the only parent out of 20 studied which gave consistently better crosses<sup>3</sup>. This stresses the need to evaluate materials being used by Indian breeders in combination with Cuba 19 and other similar populations.

For the development of superior combining lines, to be used in the production of high yielding hybrids or synthetic varieties, greater success is likely to result from the population, which show substantial heterosis incrosses and have high frequency of favourable genes. The heterosis exhibited by varietal crosses is the mean of all possible inbred lines. Selected inbred lines from these varieties

would be expected to give still higher heterotic response. Hence varieties like Cuba 19 and Kisan hold good promise for the conventional hybrid programme.

Recently attempts have been made to explain the phenomenon of heterosis, at biochemical-genetic level, in terms of enhanced mitochondrial activity<sup>5</sup>. Complementations between genetically dissimilar mitochondria of the parents in the hybrid cell<sup>6,7</sup> have been postulated to give superior mitochondrial systems, i.e., more efficiently organized systems of respiration and energy conservation. Such mitochondrial interactions might in part be responsible for heterotic responses obtained in the present investigation.

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#### THE RELATIVE IMPORTANCE OF CHLOROPHYLL CONCENTRATIONS AND GREENNESS OF LEAVES INFLUENCING GRAIN YIELD IN PEARL MILLET

SINCE chlorophyll is an essential factor in the process of photosynthesis, a study of the relation between the chlorophyll concentration in the leaves and the yielding ability of the plant would be of help in the selection of superior genotypes. As early as 1933, Sprague and Curtis<sup>2</sup> found significant correlation between the chlorophyll content and shelled corn weight. Recently, Chai and Gill<sup>1</sup> have shown that spring wheat yields are associated with the amount of chlorophyll in the leaves. Usually, the visual greenness of the leaves is taken as a criterion of the relative amount of chlorophyll content. But the study of Starnes and Hadley<sup>3</sup> has clearly pointed out that the visual differences in greenness of soybean leaves do not necessarily mean that real differences in chlorophyll content exist and the types that are visually alike may or may not contain the same kinds and amounts of chlorophyll. The present study aims at presenting the relationships among the greenness of leaves, chlorophylls *a* and *b* and grain yield in pearl millet [*Pennisetum typhoides* (Burm.) S and H].

TABLE I

Correlation coefficients among grain yield and four leaf characters in pearl millet

| Character                   | Greenness of leaves | Chlorophyll <i>a</i> (mg/g) | Chlorophyll <i>b</i> (mg/g) | Total chlorophyll |
|-----------------------------|---------------------|-----------------------------|-----------------------------|-------------------|
| Grain yield per plant       | -0.0556             | 0.4230*                     | 0.4379*                     | 0.4778*           |
| Greenness of leaves         | ..                  | -0.0531                     | 0.0705                      | 0.2429            |
| Chlorophyll <i>a</i> (mg/g) | ..                  | ..                          | 0.9320*                     | 0.9911*           |
| Chlorophyll <i>b</i> (mg/g) | ..                  | ..                          | ..                          | 0.9748*           |

\* Significant at 1% level of significance.

The material comprising 50 diverse inbred lines of pearl millet was sown in 1972 following a randomized block design with three replications at the Punjab Agricultural University, Ludhiana. The visual greenness of the leaves was scored for each inbred line at the time of anthesis. As flag leaves have been reported to be the greatest contributors to grain yield<sup>5</sup>, a representative sample of fresh tissue was taken from five fully expanded flag leaves for each inbred line and chlorophylls *a* and *b* were estimated by the method suggested by MacKinney (1941). The grain yield per plant was recorded at maturity stage. Correlation coefficients were calculated among greenness, chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a* + *b*) and grain yield per plant. The grain yield per plant exhibited no correlation with greenness of leaves while it was positively and significantly associated with chlorophyll *a*, chlorophyll *b* and total chlorophyll contents in the flag leaves (Table I).

The two chlorophylls *a* and *b*, were significantly correlated with each other as also with the total chlorophyll. These three positive correlation coefficients approached unity thus indicating an almost complete association of the three variables involved. The high correlation between chlorophyll *a* and chlorophyll *b* indicated that the selection for the higher content of one will bring about a correlated response for the higher concentration of the other component.

It is interesting to note that the greenness of leaves was correlated neither with grain yield nor with the chlorophyll *a* and chlorophyll *b*. Also, the total chlorophyll concentration in the flag leaves exhibited a low correlation with the visual greenness of the leaves. Starnes and Hadley<sup>4</sup> have suggested that several factors like thickness of the leaves, compactness of the leaf tissue, amount of

carotenoids and xanthophylls and the ratio of the two major chlorophylls present could be responsible for difficulty in the visual estimation of chlorophyll content in leaves. The present study, therefore, emphasizes the actual estimation of the chlorophyll concentration in the leaves rather than to consider the greenness of the leaves to be an indication of high low chlorophyll content. The study also brings out the importance of this physiological factor in the breeding programmes aimed at selecting the plants with higher yield potential.

Department of Plant Breeding,  
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Ludhiana, December 27, 1974.

P. S. PHUL.  
S. K. GUPTA.  
K. S. GILL.

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#### PARASITES, PREDATORS AND DISEASES OF LARVAE OF *DIACRISIA OBLIQUA* WALKER (LEPIDOPTERA: ARCTIIDAE) ON SOYBEAN\*

DURING September-November of 1973 and 1974, the larvae of *Diacrisia obliqua* Walker infesting soybeans in Jabalpur area were found attacked by parasites, predators and a fungal disease, despite the fact that the larvae are densely hairy. The parasites were two braconids, *Apanteles obliquae* Wilkinson and *Apanteles* sp. (*glomeratus* group), and five sarcophagids, *Sarcophaga misera* Walker, *Sarcophaga* sp. nr. *karkauensis* Baranov, *Goniophthalmus halli* Mensil, *Carcelia corvinoides* Wulp and *Sarcophaga* sp. The predatory bugs associated with the colonies of young larvae were two reduviids, *Rhinocoris*

*fuscipes* Fabricius and *Scadra annulipes* Reuben and two pentatomids, *Cantheconidia furcellata* Wolff and *Andrallus spinidens* Fabr. The fungus, *Spicaria rileyi* (Farlow) wiped out several colonies of young larvae of the insect. All the above excepting *A. obliquae* are recorded for the first time on the insect. The parasitism was 29.14% in 1973 and 17.57% in 1974. In both years, *A. obliquae* was predominant.

*Apanteles obliquae* was described as a new species from *D. obliqua confusa* Butl. in India (Wilkinson, 1928). Lal (1960) made biological observations on *A. obliquae*, while Kalra *et al.* (1967) recorded *Drino* sp. (Tachinidae) on the larvae in India.

*Apanteles obliqua* selected 3 to 9 days old larvae of the first and second instars for oviposition. The parasite grubs emerged from the fourth and fifth instar larvae which were retarded in size and died in 3 to 4 days of the emergence of the parasite grubs. On the other hand, *Apanteles* sp. selected for oviposition, older larvae (9 to 13 days age) which were in the late second and third instars. The parasitic grubs emerged from the full-grown host larvae which were active and suffered no apparent ill-effects and died in 3 to 4 days of parasitic grubs emergence.

The sarcophagid flies appeared distinct in their behaviour. In some cases, solitary pupae of the flies were found in the larval body which was evidently hollowed as a tunnel for the pupae. In others, the maggots cut their way out from the dorsal side of the thorax of the host larvae for pupation outside. In still other cases, the maggots emerged out of the host-body by consuming it entirely and leaving behind hairs only. One to 9 maggots emerged from a host-larva.

The predators preferred young larvae of *Diocrisia obliqua*. An adult of *Rhinocoris fuscipes* killed 1 to 3 larvae per day during the life period of 21 to 29 days in the laboratory.

The parasites and predators were identified by the British Museum, London, and the pathogens by the University of California, Berkeley, U.S.A.

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J.N. Agricultural University, G. A. GANGRADE.  
Jabalpur, M.P., India,  
December 27, 1974.

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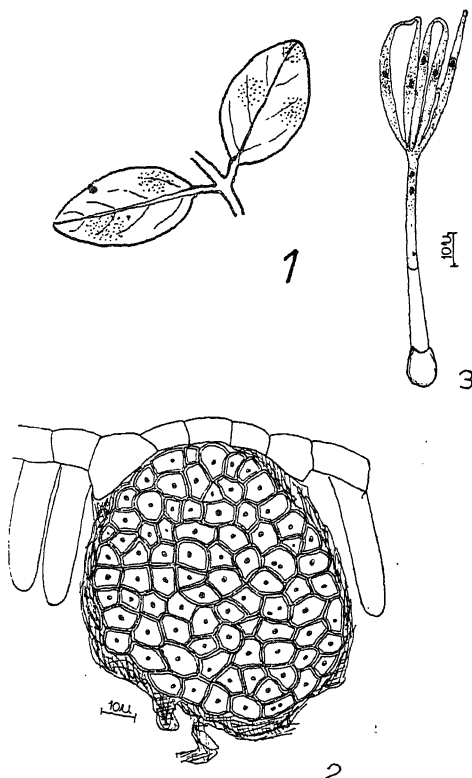
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## A NEW SPECIES OF BURRILLIA FROM INDIA

WHILE collecting smuts near Bombay during the monsoon of 1972, the author encountered an interesting leaf smut of *Hygrophila serpyllum* Anders, growing in marshy places which on examination proved to be an undescribed species of the genus *Burrillia* Setchell. In *B. anomala* Crowell, *B. narasimhanii* Thirum. and Mundk., and *B. ajrekari* Thirum., the spores are not firmly united to form compact balls but are packed in a crustose layer<sup>1</sup>. In the species under consideration all the cells of the spore ball are fertile and are firmly united to form compact spore balls. Because of these differences in the structure, composition of the spore balls and as there is no record of *Burrillia* on any species of *Hygrophila*, the present species has been regarded as new and is designated as *Burrillia kamatii*.

*Burrillia kamatii* Thakur, Sp. Nov. (Figs. 1-3)

Sori follicoli, sub-nigri, rotundati, eminentes, dispositi plus minusve regulariter in maculis luteas. Maculae irregulariter rotundae, diametientes 2-6 mm, saepe coalescentes ternese vel quaternae. Sporarium



FIGS. 1-3. *Burrillia kamatii*. Fig. 1. Leaves showing leaf spots. Fig. 2. Section through spore ball. Fig. 3. Germination teleutospore.

massae fusce brunnaeae plus minusve rotundae vel quaternae, dispersae in mesophyllum sub epidermide, sat firmiter unitae, cellulis sterilibus nullis in centro vel inter sporas. Sporarum massa circumdata textu hyphali pseudoparenchymatic fusco densitatis inequalis, in quo sporae peripherales nonnumquam sunt infexae, magnitudinis,  $120-145 \times 90-130 \mu$ . Sporae subglobosae vel angulares ob compressionem, paritibus tenuibus, pallide cinnamomo-luteae, leves,  $10.2-17 \mu$  diam. (medietate  $13.9 \mu$ ), germinantes per promycelium breve 1-2 septatum ornatum terminali verticillo sporidiorum 4-7. Sporidia fusiformia, latiora ad basim nonnumquam conjugantia bina situ.

On leaves of *Hygrophila serpyllum* Anders, Kandala, Maharashtra State, 26 June 1972. S. B. Thakur; type deposited at C.M.I., Kew, and at A.M. Herbarium, Poona-4, under No. IMI 178884 and AMH 1905 respectively.

The author is indebted to Dr. P. S. Gharase, Head of the Biology Department, Ruparel College and to Prof. M. N. Kamat, Head of the Mycology Department, M.A.C.S., Poona, for suggestions and encouragement.

Department of Botany, S. B. THAKUR,  
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#### FALSE SMUT OF RICE—AN AIRBORNE DISEASE

FALSE smut disease of paddy also known as Green smut caused by *Ustilaginoidea virens* (Cke.) Tak., was first reported by Cooke (1878) from Tinnevelly in Tamil Nadu State, India. Butler (1918) suggested that infection might be occurring at the flowering stage. Galloway (1936) produced evidence to show that the infection was neither soil-borne nor seed-borne. Raychaudhari (1946) suggested that two types of natural infections may be involved, one taking place at the early stage of flowering and the other after grains were almost mature. Successful artificial inoculation was not reported until Yoshino and Yamamoto (1952) and Ikegami (1960) found that they could cause infection with a suspension of chlamydospores of the fungus injected into the leaf sheath cavity at the booting stage of the growing paddy plants. Hypodermic method of injection of chlamydospores carried out by us during two consecutive *kharif* seasons met with no success.

We have, however, successfully infected the panicles of the flowering paddy plants by applying suspensions of fungal chlamydospores into the

fertilized and unfertilized ovaries with camel hair brush.

*Infection after fertilization.*—Individual fertilized ovaries were inoculated by applying suspension of chlamydospores of this fungus with the help of a camel hair brush. The inoculated panicles were then covered with polythene bags and the plants placed in the glass house at the Rice Research Station, Karjat (M.S.) for observation. Initial infection was observed in the form of spore balls in between the glumes covered by a membrane at the end of 8 days of inoculation. The membranes were seen to burst as a result of further growth of the spore balls; the colour of the wall changing to orange-yellow and finally to greenish black. The varieties of paddy which showed successful infection were R-24, Igatpuri 256-2 and T.N. 1 (Fig. 1).

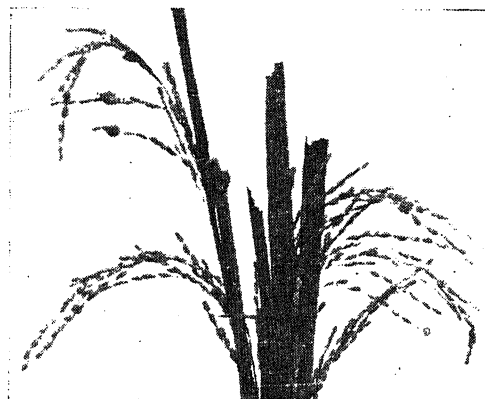


FIG. 1. Artificially inoculated plants.

*Infection before fertilization.*—The ovaries which were inoculated before fertilization invariably remained empty without any visible signs of infection. When mounted under microscope, they revealed the presence of fungal mycelium interwoven with anthers and stigmas along with chlamydospores. Thus it appears that if the infection took place before fertilization of the flowers, most of the inoculated glumes would remain sterile without any visible sign of infection.

The percentage of infection obtained in the artificially inoculated flowers at Karjat was as high as 80% taking into consideration both types of artificial infection. Similar successful infection was also obtained on R-24 variety grown on the experimental field at M.A.C.S., Poona, following the same inoculation technique as detailed above. All precautions were taken to avoid natural infection.

*Seed inoculation.*—Surface sterilized seeds of paddy R-24, smeared with chlamydospores and sown in the sterilized soil in pots did not show any infection. These findings are in agreement with



those of Galloway (1936) who reported that the mode of infection was neither soil-borne nor seed-borne. Histopathology of various parts bearing diseased grains such as stem, stalk and inflorescence revealed that the infection was not systemic. Aero-biological studies carried out at the Rice Research Station, Karjat (M.S.), during the rice growing season (June–September) further revealed that chlamydospores of this fungus could be trapped about one week before the appearance of field infection, and the concentration of such spores so trapped increased progressively at the approach of the flowering period.

These observations indicate that false smut of paddy may be an airborne disease. Primary infection may originate from the chlamydospore balls hibernating in the soil, which, under favourable conditions of environment may germinate producing large quantities of secondary conidia, that can be carried by wind and rain drops and infect the panicles of paddy plants at the 'boot' stage, very similar to what was reported by Mundkur (1943) in respect of Karnal bunt of wheat incited by *Neovossia indica* (Mitra) Mundkur.

The authors are thankful to the Indian Council of Agricultural Research, New Delhi, for financing the project and to Prof. M. N. Kamat, Head of the Mycology and Plant Pathology Department, for his keen interest taken during this investigation and making valuable suggestions and to Dr. G. B. Deodikar, Director of the Institute, for providing laboratory facilities.

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# INSECTICIDAL CONTROL OF THE SUGARCANE SHOOT BORER, *CHILO INFUSCATELLUS* SNELLEN. (LEPIDOPTERA: CRAMBIDAE)

## I. Foliar Spray

AMONG the pests that are injurious to sugarcane in Mandya District of Karnataka, the shoot borer, *Chilo infuscatellus* Snellen, is the most important. Two experiments were conducted during 1970–72 to evaluate the efficacy of some newer chemicals against this pest and the results are presented here.

The experiments were laid out in a randomised block design with four replications. The net plot size was 57.6 sq. m. (8.0 × 7.2 m). The variety used was CO 419. The chemicals (*vide* Table I) were applied four times as sprays at 0.2% strength from the fifth week after planting, at fortnightly intervals at the rate of 500 litres of spray fluid per hectare with a compressed air sprayer. Observations were recorded on the total number of tillers and the number of dead-hearts at fortnightly intervals and the percentage of dead-hearts was worked out, for the comparison of the efficacy of the insecticides. The observations were terminated after the completion of four months with the cessation of pest activity.

TABLE I  
Mean percentage of dead-hearts under different treatments

| Treatment                       | Mean percentage of dead-hearts |         |
|---------------------------------|--------------------------------|---------|
|                                 | 1970–71                        | 1971–72 |
| monocrotophos (Azodrin EC 60)   | 3.1                            | N.T.    |
| monocrotophos (Nuvacron EC 40)  | N.T.                           | 4.7     |
| carbofuran (Furadan WP 75)      | 2.5                            | 4.3     |
| trichlorphon (Dipterex WP 95)   | N.T.                           | 5.1     |
| diazinon (EC 20)                | 4.4                            | N.T.    |
| phosphamidon (Dimecron EC 100)  | 4.1                            | 6.6     |
| formothion (Anthio EC 100)      | N.T.                           | 7.5     |
| endrin (EC 20)                  | 3.1                            | N.T.    |
| endosulfan (Thiodan EC 35)      | 4.1                            | 3.7     |
| chlorfenvinphos (Birlane EC 24) | 5.9                            | N.T.    |
| Control                         | 7.8                            | 8.4     |
| F-Test                          | significant at 5% level        |         |
| C.D.                            | 2.8                            | 3.2     |
| N.T.                            | Not tested                     |         |

The results of the experiments are given in Table I. It may be concluded from the above data that for the control of the sugarcane shoot borer, spraying four times of carbofuran, endosulfan, endrin, monocrotophos, trichlorphon and diazinon at 0.2% at fortnightly interval at the rate of 500

litres of spray fluid per hectare commencing from the fifth week after planting can be recommended.

In terms of active ingredient, carbofuran and endosulgan can be applied at 1.0 kg. a.i. per hectare.

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Bangalore, September 4, 1974. H. K. SANGAPPA.

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### AN EMS INDUCED "VEGETATIVE MUTANT" OF RICE (*ORYZA SATIVA* L.)

In the course of mutation studies, a new type of mutant was isolated in the  $M_2$  generation of a locally adapted rice variety, "Tilakchandani" treated with 0.6% EMS. The mutant had branched rachis and was devoid of reproductive parts. The rachis was partially exerted and in a few cases had one or two abnormal lemma or palea-like structures on the tip (Fig. 1). Since the mutant lacked reproductive

organs and the only way of its maintenance was through vegetative reproduction, it was named as "Vegetative mutant". This mutant had erect, long, broad and thick leaves with hard texture and low senescence. There were well-developed adventitious roots upto third node.

In the  $M_2$  segregating progeny, 35 plants were normal and 6 were of mutant type which was in agreement with the monofactorial segregation of 3 normal : 1 mutant. From the  $M_3$  segregating progenies 30 normal plants were taken at random and  $M_4$  progenies were raised. Out of 30  $M_4$  progenies, 19 progenies segregated for the mutant character and 11 progenies did not segregate. The segregation pattern of  $M_4$  progenies was in the ratio of 2 segregating to 1 non-segregating which corroborated to monofactorial inheritance observed in  $M_2$  generation (Table 1).

TABLE I

*The segregation pattern of the vegetative mutant*

| Generation | Plants/<br>Progenies      | Number | $\chi^2$ values                  | P values  |
|------------|---------------------------|--------|----------------------------------|-----------|
|            | Normal plants             | 35     |                                  |           |
| $M_2$      | Mutant plants             | 6      | 2.349<br>(assuming<br>3:1 ratio) | 0.10-0.25 |
| $M_4$      | Segregating progenies     | 19     | 0.150<br>(assuming<br>2:1 ratio) | 0.50-0.75 |
|            | Non-segregating progenies | 11     |                                  |           |

Each of the segregating  $M_4$  progeny also segregated in the ratio of 3 normal to 1 mutant.

From the breeding behaviour of  $M_2$  and  $M_4$  progenies, it appears that the vegetative mutant is a recessive and a monogenic character.

The authors express their sincere thanks to the Director, Research and Dean, Agriculture, for their interest in the present study.

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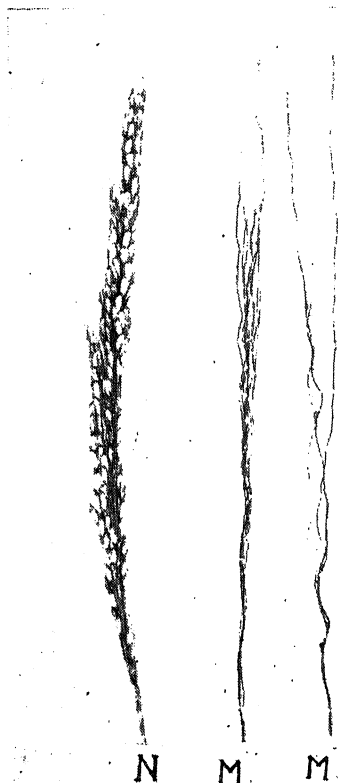


FIG. 1. N—Normal panicle, M—Panicle of Vegetative mutant.

## SHORT SCIENTIFIC NOTES

### *Secchium edule*—A New Host of *Glomerella cingulata*

Green fruits of *Secchium edule* locally known as *Chou-chou* or *seemé-badane* is a common vegetable. In January 1972, at Regional Research Station, Mudigere, rotting of green fruits of *Chou-chou* was observed on several vines. Initial symptoms appear as minute, brown water soaked, sunken spots which latter turn to dark brown in colour. Often several spots coalesce together to form a large patch, at times, covering an entire side of the fruit. The affected tissue becomes soft as the rotting sets in. In advanced condition, profuse mycelial growth is seen which turns black when fruiting bodies develop. Isolations from the affected regions yielded a *Colletotrichum* sp. which proved pathogenic when inoculated artificially. The pathogen has been identified as *Glomerella cingulata* (IMI 166177).

The fungus has been reported on several plant species from India, but, there is no record of this fungus on *Secchium edule* and hence this is a new host record.

Grateful thanks are due to Director, Commonwealth Mycological Institute, Kew, England, and Dr. Hawksworth of the same Institute, for the identification of the fungus.

Regional Research Station, T. B. ANILKUMAR.  
University of Agril. Sciences, S. VISWANATH.  
Mudigere, Karnataka,  
April 18, 1974.

### A New Virulence of Stem Rust of Wheat Attackin Choti Lerma

A stem rust sample on wheat (HD 4513) received from Wellington (Nilgiris) on analysis yielded race 34. However, a susceptible type pustule was observed on Sharbati Sonora which otherwise is resistant to type race 34. Susceptible type pustule from Sharbati Sonora was isolated and further raised on Agra local wheat for race analytical studies. The test isolate on analysis yielded race 40 as judged by its infection types produced on international differentials<sup>1</sup> of stem rust. The test isolate, however, was found to differ from type race 40 in its infection types produced on auxillary differentials, viz., Charter, Yalta and E 535 being resistant to type race 40 and susceptible to the test isolate.

Each of the single spore cultures, originating from susceptible type pustule on charter, was separately analysed. It was observed that each

single spore culture produced the infection types similar to type race 40 on the international differentials but differed from the type race in infection types produced on the auxillary differentials as stated above.

The test isolate and the type race 40 were further compared on 10 wheat cultivars, viz., UP 215, UP 319, WG 357, NI. 5439, HD 4502, HS 1138-6-4, Kalyansona, Moti, Sharbati Sonora and Sonalika. It was observed that all the wheat cultivars mentioned above were susceptible to test isolate and resistant to type race 40. Thus it is evident that the test isolate is more virulent than the type race 40.

In order to assess the real impact of the test isolate of race 40, wheat cultivars such as HD 2009, Choti Lerma, WL 208 and Zoafrane known to be resistant to all the virulences of stem rust and certain promising cultivars such as HB 117-107 and Safed Lerma were also tested. All these cultivars were found to be susceptible.

A special mention should be made of Choti Lerma, a variety being most promising and resistant to all virulences of stem rust occurring in India till recently, was recommended for cultivation in Nilgiri and Palni hills in order to cut down the stem rust inoculum at the source. Now Choti Lerma being susceptible to test isolate (race 40-A) would require the revision of this recommendation. Attempts should now be made to incorporate resistance against race 40-A in Choti Lerma by back crossing it with resistant donor against race 40-A.

Plant Pathological S. K. SHARMA.  
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### *Tetraneura radiculicola* Strand (Homoptera: Aphididae) A New Pest of Rice Seedlings

During the month of January, 1974, a few dry nursery beds of paddy were raised at the Regional Research Station of the University of Agricultural Sciences at Mandya. The seedlings in patches appeared stunted and exhibited leaf yellowing

symptoms. There was no pest incidence on the portion of the seedlings above-ground. Examination of the root of the plant along with the soil, revealed the presence of a number of aphids attached to the roots. These aphids were later on identified as *Tetraneura radiculicola* Strand.

Hille Ris Lambers<sup>1</sup> in his description of a new subgenus and new species of *Tetraneura* Hartig, 1841 has reported that *Tetraneura radiculicola* has been recorded on *Imperata arundinacea* (Kalimpong, W. Bengal) and on grass roots (Assam) in India.

This is the first record of *Tetraneura radiculicola* from South India and on rice.

The author is grateful to Dr. S. Kanakaraj David, Coimbatore, for identifying the aphid species.

University of Agril. Sciences, P. S. RAI.  
Regional Research Station,  
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#### *Prunus bokhariensis* Royle; A New Host for *Trichothecium roseum* (Persoon) Link ex Fr.

During a survey of the storage rot of fruits in the local markets in May-June, 1973 the authors noticed a fruit rot disease of *Prunus bokhariensis* Royle (commonly known as Alubukhara). Disease starts as small light brown spots and on maturity becomes dark brown to black with pink to orange coloured fungal mass seen in the central region. The pathogen was isolated and axenic culture obtained and identified as *Trichothecium roseum* (Persoon) Link ex Fr.

On Potato-dextrose-agar medium, the colony is white in the beginning and turns pink, finally becoming pinkish-orange at maturity. Growth is floccose. Conidiophores hyaline, branched, simple, septate, swollen at the tip; conidia hyaline, ovate or pear shaped with or without septation, usually one septate, borne acrogenously, measure  $12-28 \times 8-12 \mu$ , average  $16 \times 10 \mu$ .

Pathogenicity tests on fruits were established successfully. It was found that *T. roseum* is a wound parasite. Cross inoculations on *Malus sylvestris* gave positive results. The optimum temperature for spread of disease was found to be  $28^\circ\text{C}$ . There is no record of *T. roseum* on any *Prunus* fruits and this is the first report. The culture of the pathogen has been deposited in C.M.I., Kew, England, IMI No. 172968:

Authors are thankful to Dr. G. P. Agarwal, Head, Department of Postgraduate Studies and Research

in Botany, University of Jabalpur, for encouragement and laboratory facilities and to the Director, C.M.I., Kew, England, for help in identification of the species. Junior author (H. C. A.) is also thankful to the University Grants Commission for the award of Junior Research Fellowship.

Dept. of Postgraduate S. K. HASIJA.  
Studies and Res. in Botany, H. C. AGARWAL.  
University of Jabalpur,  
Jabalpur 482 001, May 1, 1975.

#### A Root Rot of Crucifers Incited by *Pythium butleri*

A root rot of cabbage (*Brassica oleracea* L. var. *capitata*) and cauliflower (*B. oleracea* L. var. *botrytis*) appeared in the cultivated fields of Varanasi, U.P., and the infection ranged between 12-15% often reaching 25% in the crop stand. Reduction in growth vigor resulted in bearing undersized heads from incipient or mildly infected plants. The roots and mesocotyl were attacked at the soil level, producing damping-off in the nurseries and collar and root rots in the field. The disease symptoms initiated as slight rolling or drooping of the lower leaves with retarded growth and loss of turgidity, the roots showing a blackish brown discoloration. A soft rot ensued in the roots and the bark sloughed off 45-50 days after transplantation.

The pathogen was isolated from the infected roots on potato dextrose agar (PDA), corn meal agar (CMA) and oats meal agar (OMA), which consistently yielded a species of *Pythium* Pringsheim. The coenocytic hyphae were hyaline, delicate and branched,  $4-9 \mu$  in diam. with sparse septation in aging cultures. Zoosporangia were lobulate, thin-walled, lateral, digitately elongated, developing a vesicle in which the protoplasm migrated. Motile zoospores released after vesicular rupture were 20-30 or more, biciliate, bean-shaped, slightly depressed at the hilum, bearing 2 long flagella and  $7-11 \mu$  in diam. after coming to rest. Sexual organs developed on PDA, CMA and OMA. The oogonia were lateral or intercalary, spherical to globose, thin-walled, measuring  $17.5-30 \mu$  (avg.  $22.5 \mu$ ), while the antheridia were terminal or intercalary or hypogynous, knob-shaped, measuring  $9-11.5 \times 6.5-8.5 \mu$ . The oospores were round, smooth, hyaline to light yellowish when fully mature, thick-walled never filling the oogonium completely, measuring  $15.5-26.5 \mu$  (avg.  $20.5 \mu$  India) and germinated by a germ tube. The oospores of the pathogen developed abundantly in the cortical tissues of the roots and mesocotyl, helping its survival in the soil as a source of primary inoculum.

Inoculations with fragmented mycelium or zoospores on the root seedlings raised in sterile sand, developed disease symptoms in 7-11 days, identical to those in the field. Similar inoculations were made on other crucifers such as mustard (*Brassica campestris* L.), turnip (*B. napus* L.), knol khol (*B. caulorapa* L. var. *caulorapa*) and radish (*Raphanus sativus* L.) in which mustard became infected. A mild infection appeared in turnip and radish while knol khol was not susceptible at all. Cultural characters and morphology of the pathogen indicated its identity with *Pythium butleri* Subramaniam, to which it is referred (IMI 173180). Root and stalk rot and damping off diseases incited by this pathogen have been recorded on several economic crop plants in India, but not in cabbage and cauliflower and other crucifers, thus extending its host range from India.

The first author (SLS) expresses his gratitude to the Indian Council of Agricultural Research, New Delhi, for the award of a Senior Research Fellowship. Thanks are due to the Director, Commonwealth Mycological Institute, Kew, England, for identifying the species.

Faculty of Agriculture,  
Banaras Hindu University,  
Varanasi-5, May 2, 1975.

S. L. SINGH.  
M. S. PANGOL.

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#### A New Record of *Bacillus cereus* on the Spotted Bollworm, *Earias vittella* (F.)

In recent studies on the pests of cotton and bhendi (*Abelmoschus esculentus*), the larvae of *Earias vittella* (F.) were found infected by a bacterium in the field. The pathogen was identified as *Bacillus cereus*.

*B. cereus* has been reported on the southern armyworm (Barbers, 1938), eye-spotted bud-moth, *Spilonota ocellana* (Legner, 1973), codling moth (Stephens, 1952) and larch sawfly, *Pristiphora erichsonii* (Htg.) (Heimpel, 1954 b). But there appears to be no record of it on *E. vittella*.

While examining the bollworm infested bhendi fruits collected from the fields around Dharwar, the authors noticed some of the larvae dead inside the fruits. The body was filled with fluid; the skin was intact and the fluid emitted a putrefying smell when teased. Colour of the body was pale. The percentage of the infected larvae varied from 9 to 10. Medium-sized larvae which measured 8 mm to 11 mm were found highly susceptible.

This appears to be the first record of *B. cereus* on *E. vittella*.

Our thanks are due to Dr. Gerard M. Thomas, of the University of California for identifying the pathogen, and to Dr. S. V. Patil, Director of Instruction, Agricultural College, Dharwar, for encouragement.

Department of Entomology, T. S. THONTADARYA.  
College of Agriculture, S. N. HOLIHOSUR.  
Dharwar 580 005, I. G. HIREMATH.  
May 1, 1975.

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## REVIEWS AND NOTICES OF BOOKS

**Crystal Physics : Macroscopic Physics of Anisotropic Solids.** By Hellmut J. Juretschke. (W. A. Benjamin, Inc., Massachusetts), 1974. Pp. xvi + 200. Price \$ 19.50.

The book deals primarily with the effect of the symmetry of crystals on their macroscopic physical properties. The first four chapters give a short and clear introduction to groups, tensor analysis and crystallographic point groups in two and three dimensions. The rest of the book is devoted to an enumeration of the symmetry of various physical properties. Each chapter begins with a brief introduction to the physics of the interaction responsible for the property in question and examines the effect of symmetry. In some cases, (like the four probe measurement of conductivity) experiments for measuring the properties are discussed. Chapters 6 and 7 form a very readable account of time reversal, magnetic symmetry and transport properties. Of particular interest to people working in the field are the transformation equations of conductivity tensors given at the end of Chapter 7. The discussion of polycrystalline media in Chapter 13 is also very interesting. Problems given at the end of each chapter make the book very useful to students. The book is indeed a valuable addition to the existing literature on crystal symmetry and physical properties.

S. BHAGAVANTAM.

**Annual Review of Genetics (Vol. 8).** Herschel L. Roman, Allan Campbell and Laurence M. Sandler, Editors. (Annual Reviews, Inc., Palo Alto, California), 1974. Pp. 480. Price \$ 15.00.

Short of reading and taking notes from every paper published, the Annual Reviews service is the best way of keeping abreast in any discipline. Hence, the publication of volume 8 of *Annual Review of Genetics* will be hailed by geneticists, molecular biologists and cell biologists. Naturally not everyone will be interested in every paper, but there is something here for everybody. The chapter titles bear witness to this: Analysis of Genetic Regulatory Mechanisms; Controlling Elements in Maize; The Relationship between Genes and Polythene Chromosome Bands; Genetic Polymorphism of the Histocompatibility-2 Loci of

the Mouse; Biochemical Genetics of Bacteria; Genetics of Amino Acid Transport in Bacteria; Genetic and Antibiotic Modification of Protein Synthesis; On the origin of RNA Tumor Viruses; Pheromones as a Means of Genetic Control of Behaviour; Gene Expression in Somatic Cell Hybrids; Regulation—Positive Control, Accessory Chromosomes; Somatic Cell Genetics of Higher Plants; Fungal Genetics; Genetics of DNA Tumor Viruses; Frameshift Mutations; Genetic Analysis of the Chloroplast and Mitochondrial Genomes; The Origin of Life; and Gene Control of Mammalian Differentiation.

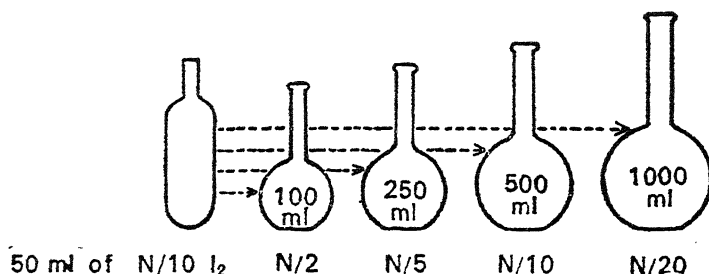
A few random comments are as follows. The paper by Drs. Beckwith and Rossow on genetic regulatory mechanisms not only brings things up to date but also points out the limitations of many of the approaches applicable to the characterization of regulatory genes and suggests the necessary criteria for defining such mechanisms. These points are further extended in the article by Drs. Englesberg and Wilcox on positive control of regulations. The biochemical aspects of both negatively and positively controlled regulation have been discussed by Drs. Gots and Benson. They have also described autoregulation, which is a major divergence from the original Jacob-Monod operon hypothesis. Dr. Halpern's article on amino acid transport in bacteria underlines the three recent areas of main emphasis and activity in the field of transport, namely, binding proteins, transport in membrane vesicles, and energization of transport. Dr. Temin's paper on RNA tumor viruses discusses the provirus hypothesis which states that these viruses repeatedly evolved from normal cellular processes that were similar in related animals because of their common ancestry, and concludes that the normal cellular process of DNA to RNA to DNA information transfer could be important in differentiation and evolution. A parallel article by Dr. Eckhart on DNA tumor viruses deals with the genes in SV 40 virus which effect transformation. And so on.

As usual, each chapter is abundantly documented, there is an excellent index by both subject and author, and a cumulative author index and an alphabetical listing of chapter titles for volumes 4 to 8.

This book is essential for every personal and institutional library.

T. RAMAKRISHNAN.

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# POLAROGRAPHIC STUDY OF Cd (II)-PYRIDINE SYSTEM IN WATER-METHANOL MIXTURES

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Department of Chemistry, Marathwada University, Aurangabad, Maharashtra

## ABSTRACT

The Cd(II)-pyridine system has been studied in (i) 0, 10, 20, 40, 50 and 70% (v/v) methanol-water mixtures and (ii) in 20% (v/v) DMF-water mixture. The coordination number ( $p$ ) calculated from the slopes of the plot of  $(-E_{1/2})_c$  vs  $\log C_L$  gave three segments which showed the existence of 1:1, 1:2 and 1:3 complex species. The value of  $p$  increased with the increase in concentration of the ligand. The method of DeFord and Hume was adopted for the calculations of the formation constants.

It was observed that (i) three complex species are formed in all the solvent mixtures and (ii) the  $\log \beta$  values remain nearly constant with the increase in the percentage of methanol from 10 to 70%. The experimentally determined  $\log \beta$  values were utilised to understand the chemical specificity of the solvent mixtures.

## INTRODUCTION

THE stability constants of metal complexes of pyridine have been the subject of study by many workers. Metal ions such as copper<sup>1</sup>, zinc<sup>2-6</sup>, cobalt<sup>6-9</sup>, nickel<sup>6-8,10-12</sup> and palladium<sup>13</sup> were investigated by some workers to examine their interactions with pyridine in aqueous as well as in non-aqueous media using different electrolytes. Cd(II)-pyridine system has been investigated in aqueous<sup>3,4,14,15</sup>, in DMF-water mixtures<sup>16</sup>, in 50% (v/v) ethanol-water mixture<sup>5</sup> and in ethylene glycol medium<sup>17</sup>.

Despite the fact that the Cd(II)-pyridine system was investigated in certain non-aqueous media, a systematic study covering a wide range of solvent mixtures is still lacking. Such a study will help us to understand the nature of binding between the metal ion and pyridine. This work was, therefore, undertaken to investigate this aspect.

## EXPERIMENTAL

Cambridge Pen Recording type polarograph with the necessary accessories was used for the measurement of half-wave potentials and the diffusion currents. All potentials were measured against the S.C.E. The capillary electrode used had the following characteristics:

$t = 3.0$  sec in 0.5 M KCl (open circuit)

$m = 2.21$  mg sec<sup>-1</sup> and  $m^{\frac{2}{3}}I^{\frac{1}{3}} = 1.56$  mg<sup>2/3</sup>sec<sup>-1</sup>

Decameter DK 03 supplied by Wissenschaftlich-Technische Werkstätten GmbH, Weilheim/Obb-Germany was used for the measurement of dielectric constants of the different solvent mixtures.

Cadmium (BDH, AnalaR), was used as its nitrate. Methanol (AnalaR) was purified by the method of Lund and Bjerrum<sup>18</sup>. N,N-Dimethylformamide (L.R. Merck) was purified as described by Wawzonek *et al.*<sup>19</sup>. The mercury was purified by treatment with dilute nitric acid,

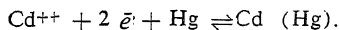
degreased with 0.1 N sodium hydroxide solution and then distilled thrice. Potassium nitrate (AnalaR, BDH) was used without any further purification. The measurements were taken in an airconditioned room where the temperature was maintained at  $25 \pm 0.5^\circ\text{C}$ .

All the solutions contained 1 mM cadmium nitrate and 0.004% Triton X-100 as a maximum suppressor. The concentration of pyridine varied from 0.103 M to 2.131 M and the ionic strength was maintained at 0.1 M by the addition of an appropriate amount of 2.0 M potassium nitrate solution. Dissolved oxygen was removed by bubbling purified nitrogen.

## RESULTS AND DISCUSSION

Though negligibly small, the  $iR$  drop corrections were taken into account and the values of  $-E_{1/2}$  were modified. These values along with the ligand concentrations are listed in Table I. The half-wave potentials were obtained from the log plots. The diffusion currents,  $i_d$ , were corrected for the residual current. The shift in half-wave potentials of cadmium (II) towards more negative values in the presence of pyridine in aqueous as well as in non-aqueous media indicated complex formation between cadmium (II) and pyridine.

The slopes value of the straight lines of the log plots were in the range of 30 to 34 mV indicating that the reduction of cadmium (II) ion in the absence and in the presence of pyridine is reversible and involves two electrons. This was found to be true in every methanol-water and DMF-water mixtures. The reduction could be represented as:



The values of  $(-E_{1/2})_c$  were plotted against  $\log C_L$  for all sets of experiments (Fig. 1) where  $C_L$  is the molar concentration of the ligand. The relationships were not linear but were represented by smooth curves with three distinct segments.

TABLE I

Analysis of  $-E_1$  of Cd(II)-pyridine in methanol-water mixtures and 20% DMF-water mixture

| $E_{1/2}$ | 0     | 10%   | 20%   | 40%   | 50%   | 70%   | 20% DMF |
|-----------|-------|-------|-------|-------|-------|-------|---------|
| 0.030     | 0.580 | 0.580 | 0.578 | 0.576 | 0.572 | 0.570 | 0.586   |
| 0.103     | 0.598 | 0.597 | 0.595 | 0.595 | 0.590 | 0.585 | 0.600   |
| 0.203     | 0.607 | 0.607 | 0.608 | 0.604 | 0.601 | 0.596 | 0.614   |
| 0.309     | 0.617 | 0.615 | 0.615 | 0.610 | 0.609 | 0.602 | 0.621   |
| 0.412     | 0.622 | 0.622 | 0.620 | 0.618 | 0.614 | 0.607 | 0.628   |
| 0.515     | 0.627 | 0.626 | 0.626 | 0.623 | 0.622 | 0.612 | 0.603   |
| 0.613     | 0.634 | 0.631 | 0.630 | 0.628 | 0.627 | 0.616 | —       |
| 0.824     | 0.642 | 0.640 | 0.640 | 0.635 | 0.632 | 0.625 | 0.644   |
| 0.927     | 0.644 | 0.643 | 0.643 | 0.637 | 0.635 | 0.628 | 0.646   |
| 1.030     | —     | 0.645 | 0.645 | 0.646 | 0.638 | 0.630 | 0.649   |
| 1.133     | 0.650 | 0.648 | 0.647 | 0.644 | 0.640 | 0.633 | 0.651   |
| 1.236     | 0.653 | 0.650 | 0.650 | 0.648 | 0.643 | 0.635 | 0.652   |
| 1.442     | 0.653 | 0.655 | 0.656 | 0.652 | 0.646 | 0.639 | 0.656   |
| 1.545     | 0.658 | 0.659 | 0.658 | 0.655 | 0.648 | 0.642 | 0.659   |
| 1.648     | 0.660 | —     | 0.660 | 0.657 | 0.650 | 0.645 | 0.661   |
| 1.854     | 0.655 | 0.663 | 0.663 | 0.660 | —     | 0.648 | 0.664   |
| 1.880     | —     | —     | —     | —     | 0.656 | 0.650 | —       |
| 2.061     | 0.668 | 0.666 | —     | —     | —     | —     | —       |
| 2.131     | —     | —     | —     | —     | 0.660 | —     | —       |

The coordination numbers ( $p$ ) calculated from the slopes of these segments were about 1, 2 and 3 which showed the existence of 1:1, 1:2 and 1:3 complex species respectively. The value of  $p$  increased with the increase in concentration of the ligand.

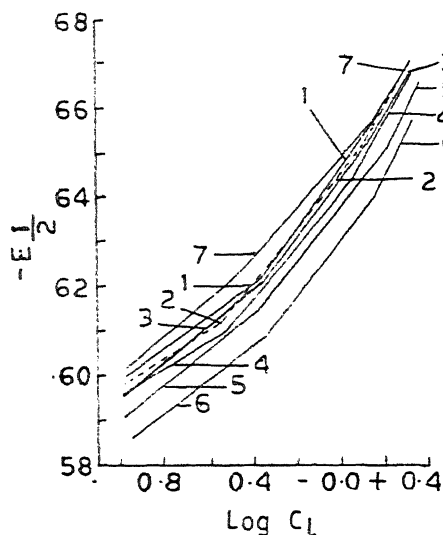


FIG. 1. Plots of  $-E$  vs  $\log C$  in various methanol-water mixtures: 0% methanol (1), 10% methanol (2), 20% methanol (3), 40% methanol (4), 50% methanol (5), 70% methanol (6) and 20% DMF (7).

The method of DeFord and Hume<sup>20</sup> was adopted for the calculations of the formation constants. The formation constants were obtained by plotting the  $F_j(L)$  functions against the ligand concentrations and extrapolating the respective curves to zero concentration of the ligand for the system in aqueous medium (Fig. 2). Similar plots have been obtained in the other cases.

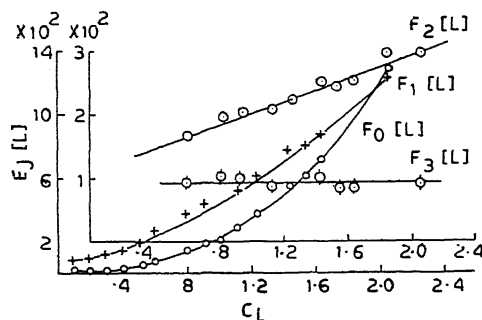


FIG. 2. Plots of  $F_j[L]$  in 0% methanol.

The  $\log \beta$  values for Cd(II)-pyridine systems in various solvent mixtures are presented in Table II. The accuracy of the  $\beta$  values in both aqueous and non-aqueous media was observed to be of the order of  $\pm 2$  for  $\beta_1$ ,  $\pm 5$  for  $\beta_2$  and  $\pm 4$  for  $\beta_3$ . The literature values, wherever available, at an ionic strength of 0.1 M ( $KNO_3$ ) are also given in the same table. It could be seen from the

TABLE II  
Cd(II)-pyridine system in water and methanol-water mixture  
 $\mu = 0.1$  (KNO<sub>3</sub>) Temp. =  $25 \pm 0.5^\circ \text{C}$

| Mol. fraction of methanol % | 0  | 10    | 20    | 40    | 50    | 20% DMF |
|-----------------------------|--|-------|-------|-------|-------|---------|
| Wt. fraction of methanol %  | —  | 7.91  | 15.82 | 31.67 | 39.57 | 55.40   |
| $1/\epsilon \times 10^{-3}$ | 12.73  | 13.51 | 14.38 | 16.67 | 18.01 | 21.74   |
| $\log \beta_1$              | 1.48<br>1.40 <sup>(14)</sup><br>1.36 <sup>(14)</sup>                         | 1.48  | 1.48  | 1.54  | 1.54  | 1.48    |
| $\log \beta_2$              | 2.04<br>1.95 <sup>(14)</sup><br>1.86 <sup>(14)</sup><br>2.14 <sup>(15)</sup> | 1.90  | 1.90  | 1.70  | 1.85  | 1.30    |
| $\log \beta_3$              | 1.95<br>2.27 <sup>(14)</sup><br>1.90 <sup>(14)</sup>                         | 2.00  | 2.00  | 1.98  | 1.90  | 1.74    |

literature values that the agreement between our  $\beta_1$  and  $\beta_2$  values and those reported by Morinaga<sup>14</sup> and Sharma and Gaur<sup>4</sup> is satisfactory. Our value of  $\beta_3$  is closer to that reported by Sharma and Gaur<sup>4</sup> than that of Morinaga<sup>14</sup>.  $\beta_1$  value for Cd(II)-pyridine system obtained by EMF measurements is 17.8 as reported by Desai and Kabadi<sup>21</sup>. The polarographic values are, however, higher than the EMF values.

Pyridine is a very weak base, since it derives its basicity from the unshared electrons of the nitrogen atoms in a plane trigonal  $sp^2$  orbital. Considering its low basicity, however, it is capable of forming a stable complex with Cd(II). This is probably due to the additional  $\pi$ -bonds ( $M \rightarrow L$ ) between the 'completely filled  $d$  orbitals of Cd(II) and the  $\pi$ -electron sextet of pyridine.

$M \rightarrow L$   $\pi$ -bonding could be examined by comparing the  $\beta$  values of Cd(II) and Zn(II) (literature values 4) with pyridine. These values at 0.1 M (KNO<sub>3</sub>) ionic strength are  $\log \beta_1 = 0.90$  and  $\log \beta_2 = 1.53$ . The  $\beta$  values for Cd(II)-pyridine system are higher than those of Zn(II)-pyridine system. The stability of these complexes depends on the relative effects of both  $\sigma$ - and  $\pi$ -bonds. The strength of  $M \rightarrow L$   $\pi$ -bond is governed by the mobility of the free  $d_e$  electrons of the central atom. The  $\pi$ -bonds in the Cd(II)-complexes are stronger than those in Zn(II) complexes since ionisation potential of cadmium is lower than that of zinc if as a first approximation the mobility of the electrons in the ions is a measure of ionization potential of the metals.

#### EFFECT OF DIELECTRIC CONSTANT ON $\beta$ VALUES

The  $\log \beta$  values remain nearly constant with the increase in the percentage of methanol from

10% to 70%. The plots of  $\log \beta_i$  vs  $1/\epsilon$  (Fig. 3) show:

- a straight line relationships for  $\log \beta_1$ ,
- that  $\log \beta_2$  values remain nearly constant upto 50% of methanol-water mixture and then register a fall, and
- that in the case of  $\log \beta_3$  values a near linearity is observed with respect to  $1/\epsilon$  values over the entire range of solvent mixtures investigated in the present work.

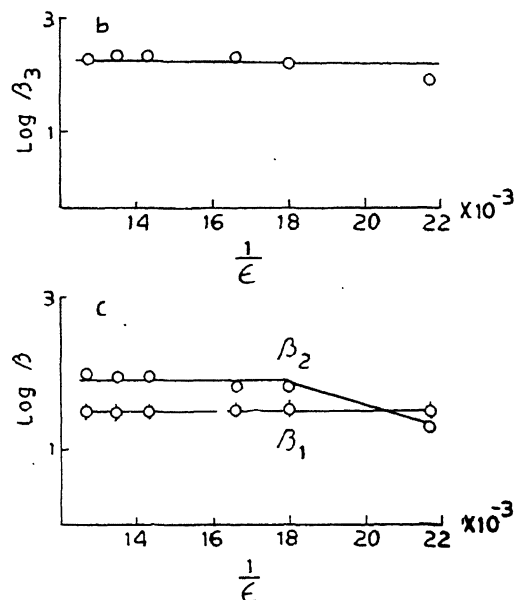


FIG. 3. Plots of  $\log \beta_1$ ,  $\log \beta_2$ , and  $\log \beta_3$  vs  $1/\epsilon$  in various methanol-water mixtures.

The near constancy of  $\log \beta_1$ ,  $\log \beta_2$  and  $\log \beta_3$  values with the sole exception of one  $\log \beta_2$  value supports the argument put forward regarding the back donation of electrons from the metal to the pyridinium nucleus. The predominance of the covalent nature of the metal-ligand bond in the 1:1 complex will not show any significant change in the  $\log \beta_i$  values with respect to changes in dielectric constant.

The constancy of the  $\log \beta$  values at various percentages of methanol shows that the solvent methanol as such is not playing any part in the reaction by which complexation occurs. This may be due to the fact that pyridine is a stronger base than methanol. Further, the mixtures of pyridine, water and methanol are a complex system due to the possibility of hydrogen bonding between water and pyridine and also between methanol and pyridine. Adam<sup>22</sup> has reported that: (i) the water molecules approach the nitrogen lone pair of electrons in such a way that the hydrogen bonded system O—H...N is linear and (ii) the results of methanol-pyridine system are parallel to those of water pyridine system, the only difference being in the orientation of the methyl groups. Either the carbon atom of the methyl group is in the plane of pyridine ring or perpendicular to it. Since it is not possible to know the concentration of water molecules and methanol molecules, hydrogen bonded to pyridine separately, no quantitative conclusions can be drawn from the observed results.

The system was also investigated in 20% (v/v) DMF-water mixture, the  $1/\epsilon$  value of which is  $13.52 \times 10^{-3}$ . The  $\log \beta$  values in methanol-water mixture corresponding to  $1/\epsilon$  values of  $13.52 \times 10^{-3}$  were obtained from the plots of  $\log \beta_i$  vs  $1/\epsilon$  (Fig. 3). The comparative data are given in Table III. In this case a discrepancy

TABLE III

| $\log \beta_i$ values in 20% (v/v) DMF-water mixture having $1/\epsilon = 13.52 \times 10^{-3}$ | $\log \beta_i$ values in methanol-water mixture having $1/\epsilon = 13.52 \times 10^{-3}$ |
|---|--|
| $\beta_1 = 1.30$  | 1.50   |
| $\beta_2 = 2.00$  | 1.90   |
| $\beta_3 = 1.60$  | 2.00   |

between the two sets of values was observed. The difference in specific chemical effects of the two organic solvents despite identical dielectric constant of their mixtures is probably responsible for the observed discrepancy. The possibility of hydrogen bonding will be greater at the higher concentrations

of pyridine ions which may have resulted in the large discrepancy in  $\log \beta_3$  as compared to  $\log \beta_1$  and  $\log \beta_2$ .

## ACKNOWLEDGEMENTS

The author is greatly indebted to Dr. D. D. Khanolkar, Professor and Head of this Department and Dr. D. V. Jahagirdar, Reader, for their numerous suggestions and valuable discussions during this investigation. Thanks are also due to Dr. A. K. Sundaram, B.A.R.C., Bombay, for his help and encouragement.

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## IMPACT OF SUPERPLASTIC STATE ON FATIGUE OF AN ALUMINIUM BRONZE

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WE report here two unusual features observed during low cycle fatigue of an aluminium bronze (referred to by the trade name ALRONZE) containing by weight per cent, besides copper, 9.5 aluminium and 4.0 iron. This alloy, in the hot rolled condition, has been found to exhibit superplastic behaviour during slow tensile deformation at elevated temperatures<sup>1</sup>. At 800°C and strain rate of  $6 \times 10^{-4} \text{ sec}^{-1}$ , round tensile specimens made from hot rolled plate of this alloy exhibit elongations in excess of 700%. The fine scale microstructure (average phase size  $\sim 10 \mu\text{m}$ ) in this situation is characterised by the presence of an  $\alpha$  phase, which is a face centred cubic solid solution of aluminium in copper, ( $\alpha + \gamma_2$ ) eutectoid micro-constituent in which  $\gamma_2$  is an intermetallic compound with the  $\gamma$  brass structure, and  $\delta$  particles of iron rich phase with the body centred cubic structure. Superplasticity in tension tests has been explained in terms of a high strain rate sensitivity of flow stress ( $\sigma$ ) of this alloy for which the strain rate sensitivity index ' $m$ ' (vide equation  $\sigma = k \dot{\epsilon}^m$  where  $k$  is a constant and  $\dot{\epsilon}$  is the strain rate) has been found to be as high as 0.6 at the conditions of temperature and strain rate mentioned above. There are negligibly few investigations<sup>2</sup> on post-formed fatigue characteristics of superplastic materials. The present investigation was therefore aimed at an examination of the alternate strain behaviour of superplastic ALRONZE. The two unusual features reported here concern the shape of the mechanical hysteresis loop and the shape instability of the test specimen when subjected to low cycle fatigue deformation in the superplastic condition.

Low cycle fatigue tests were conducted at 800°C and at room temperature using extension cycle control and a frequency of 1 cpm. Necessary modifications to the available Floor Model TT-CM-L Instron Universal Testing Machine were designed and fabricated by us<sup>3</sup> and consisted essentially of massive pull rods, a split furnace, cooling system to maintain the Instron load cell below 65°C and specimen locknuts to enable rigid holding of the specimen during push-pull fatigue. An hour-glass type geometry was chosen for the specimen with a gauge length of 3.81 mm and diameter of 3.18 mm.

The mechanical hysteresis loops recorded at 800°C and at ambient temperature (30°C) are shown in Figs. 1 and 2 respectively. The hysteresis loop in Fig. 1 corresponding to the superplastic temperature may be characterised by three features: (1) maximum stress does not differ significantly from that at zero strain; (2) extremely low stresses with the maximum being 0.65 kg/mm<sup>2</sup>; and (3) near absence of elastic deformation (the magnitude of plastic strain range  $\Delta \epsilon_p = 4.2\%$  is to be compared with that of the total imposed strain range  $\Delta \epsilon_t = 4.6\%$ ). These features may be contrasted with the corresponding ones of the hysteresis loop in Fig. 2 in which may be seen the following: (1) maximum stress is thrice as much as that at zero strain; (2) much higher stresses with the maximum being 60 kg/mm<sup>2</sup>, two orders higher than that in Fig. 1

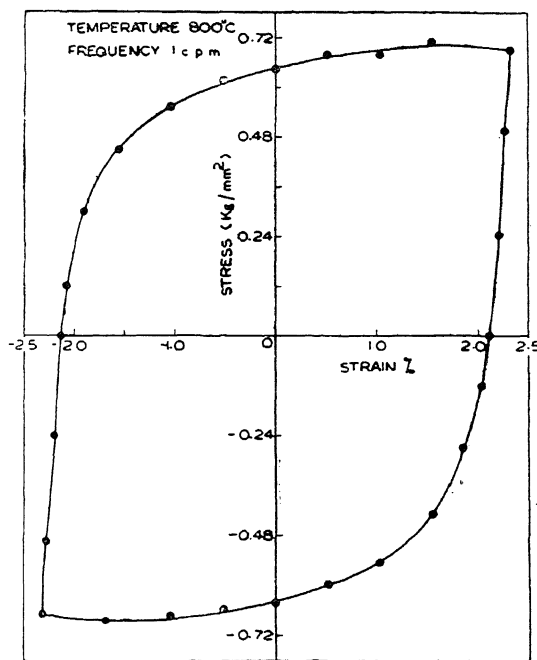


FIG. 1. Mechanical hysteresis loop at 800°C.

and (3) considerably larger elastic strain component ( $\Delta \epsilon_e = 13.4\%$  and  $\Delta \epsilon_p = 5.6\%$ ). Clearly the observed transition in the hysteresis loop at the higher temperature is a consequence of the much

higher strain rate sensitivity of flow stress at 800° C. ( $m = 0.64$ ) as compared to that at room temperature ( $m = 0.03$  as determined by us using the stress relaxation technique).

Generally, we can express the dependence of flow stress ( $\sigma$ ) on strain ( $\epsilon$ ) and strain rate ( $\dot{\epsilon}$ ) as

$$\sigma = k \epsilon^n \dot{\epsilon}^m \quad (1)$$

where  $n$  is the strain hardening exponent,  $k$  a constant and  $m$  is the strain rate sensitivity index. At ambient temperatures  $m$  is very small and the flow stress is strongly dependent on strain owing to work hardening. Thus in Fig. 2 maximum stress

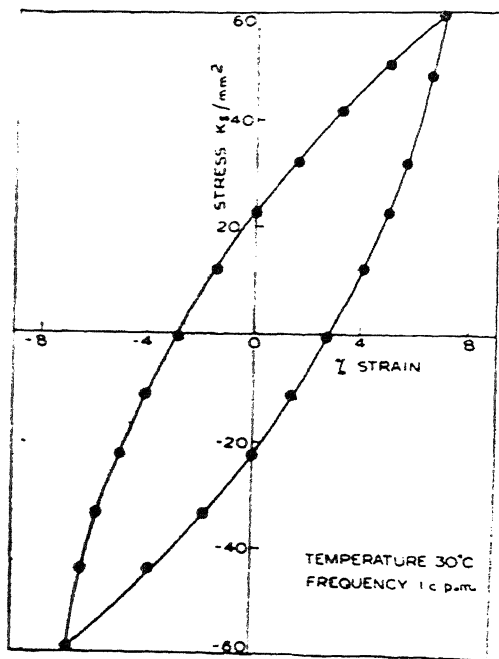


Fig. 2. Mechanical hysteresis loop at 0° C.

occurs at maximum strain. When a material is in the superplastic state characterised by negligible work hardening and high strain rate sensitivity, equation (1) essentially becomes  $\sigma = k \dot{\epsilon}^m$ . In view of our experiments having been performed on the Instron at constant cross head speed ( $v$ ) the strain rate is maximum at the commencement of the test and varies ( $\dot{\epsilon} = v/L$  where  $L$  is the gauge length of the specimen) somewhat during tension and compression of the specimen. In the superplastic state the flow stress of the alloy is

strain rate dependent as stated in the foregoing, and thus exhibits nearly maximum flow stress at zero strain (Fig. 1). The low flow stresses of superplastic alloys arise due to deformation at elevated temperatures (0.82  $T_m$  in the present instance) of extremely fine grained ( $\sim 10 \mu m$  in the present instance) microstructures. Most of the deformation is plastic in this situation owing to extremely low yield stress.

Further, during fatigue at 800° C, specimens of ALRONZE exhibited considerable shape instability in the form of specimen shortening and diameter fattening (Fig. 3). Coffin<sup>4</sup> has reported observa-

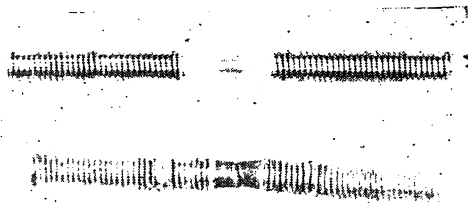


Fig. 3. Specimen instability at 800° C.

tion of similar specimen shape instability in 2S aluminium, nickel, 304 stainless steel and 1010 steel at elevated temperatures. According to Coffin's explanation, second order non-zero mean stresses, which arise because of a difference in the response of the material during tension and compression, cause superposed monotonic deformation and lead to specimen instability. It is possible that the same reason applies in the present instance with the non-zero mean stress being compressive in nature. The very low flow stress and large plasticity of the material due to the superplastic condition obviously accentuate shape instability for ever-so-small a value of the non-zero mean stress.

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## FOSSIL MOLLUSCA FROM THE SIWALIKS OF EASTERN NEPAL

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**F**OSSIL animals from the Miocene to early Pleistocene Siwaliks of southern Nepal have been formally reported only by Sharma<sup>1</sup> and informally in several Nepal Geological Survey unpublished papers. Aside from the mandible of a primitive hippopotamus, *Hexaprotodon*, found as a float occurrence near Janakpur<sup>1</sup>, all other animal fossils are from Siwalik exposures in western Nepal.

The 1974 Milwaukee Public Museum Paleontological Reconnaissance in Nepal surveyed poorly exposed Siwalik rocks in eastern Nepal, from the valley of the Arun River on the east to the valley of the Kamla River on the west. Poorly preserved molluscan fossils were collected at one locality. These specimens suggest the presence of stratigraphic units in eastern Nepal that are approximately equivalent to those that have been mapped by the Nepal Geological Survey in western Nepal.

Fossil locality MPM-N 1 (Fig. 1) is located at 26° 50' N and 86° 36' E on Survey of India topographic map J/9 (No. 72). It is on the northeastern bank of the Trijuga river, approximately 1.5 kilometres upstream of the small settlement of Gunte. The fossils were collected from a dark claystone in a sequence of irregularly alternating sandstones and claystones, dipping steeply north-westward. The locality is approximately 225 metres above sea level, and the rocks are tentatively assigned to the middle part of the poorly differentiable Siwalik series<sup>2</sup>.

The fossils reported here are two specimens of mollusc, preserved as casts, which were the germinal structures for ovoid concretions within a chaotic soft mudstone. These specimens have been deposited in the collection of the Department of Geology, Trichandra College of Tribhuvan University, Kathmandu, Nepal.

The two molluscs (Figs. 2 and 3) are difficult to identify because of their incompleteness and the nature of the preservation. The better preserved of the two belongs in the order Unionoidea, but more specific assignment is not possible with any degree of certainty. Dr. G. Shaak suggests (personal communication) that it may belong to the genus *Etheria*, Family Etheriidae; this genus has a Pliocene to Recent, Africa-Indian Ocean region distribution.

The only other area in the Nepal Siwaliks from which identifiable molluscs have been reported is the Dang Deokhur area, some 420 km to the west-northwest of locality MPM-N 1. Tshering (unpublished work) identified the gastropods *Turritella* and *Cerithium* and the pelecypods *Unio* and *Venus* from middle Siwalik gray clays exposed in the

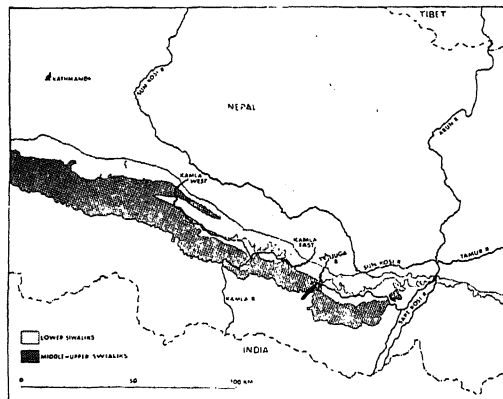


FIG. 1. Location of the fossil mollusc site, MPM-N 1. Siwalik rock distribution is indicated by the dotted pattern, based on the geologic map by Ithara et al., 1972.



drainage of the Rapti River. Unidentifiable molluscs were reported in presumed upper Siwalik rocks in the Bheri and Babai River valleys by Kayastha (unpublished work).

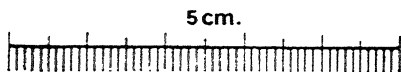


FIG. 2. Fossil mollusc from locality MPM-N 1.

As is also the case in western Nepal, the Trijuga locality produced plant remains in the form of amorphous vegetation mats, root casts and concentrations of carbonaceous material.

Locality MPM-N 1 is, by reason of the fine-grained sediment, considered to be in the middle part of the Siwalik series. Zonation of the Siwaliks is based primarily upon fossil vertebrate assemblages in the well-studied Siwalik Hills of India and Pakistan, and only secondarily upon a general increase in average grain size upward in the section with much of the upper Siwaliks being conglomeratic. In Nepal, due to the absence of stratigraphically dependable vertebrate evidence, grain size is the primary criterion for stratigraphic assignment, and this is certainly inadequate. The only reasonably detailed geologic map<sup>2</sup> of the eastern Nepal

Siwaliks places MPM-N 1 almost squarely upon a thrust fault which separates lower Siwalik rocks on the north from middle-upper Siwalik rocks on the south. While we tend to agree with the delineation of the sedimentary units, we see no evidence for such a thrust fault.



FIG. 3. Fossil mollusc from locality MPM-N 1.

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## LETTERS TO THE EDITOR

## X-RAY DETERMINATION OF THE THERMAL EXPANSION OF CERIUM TRIFLUORIDE

THE crystal structures of  $\text{LaF}_3$  and its isotypes  $\text{CeF}_3$ ,  $\text{NdF}_3$  and  $\text{PrF}_3$  have been the subject of apparently conflicting reports<sup>1-6</sup>. These rare earth trifluorides have the structure of a naturally occurring mineral tysonite, crystallizing in the space group  $P_3\text{Cl}$  of trigonal system<sup>3</sup>.

The polymorphism, structure and the unit cell parameters of these compounds at room temperature have been recently reported by Sobolev *et al.*<sup>7</sup> and Spedding *et al.*<sup>8</sup> have published the high temperature enthalpies of a number of rare earth trifluorides. The only substance of this group, for which the data on thermal expansion are available, is  $\text{LaF}_3$ . Sher *et al.*<sup>9</sup> made detailed investigations of thermal expansion on  $\text{LaF}_3$  as determined by X-ray method and also, using a dilatometer. They followed Anderson and Proctor<sup>6</sup> in reporting the value of the *c*-parameter in terms of the double hexagonal cell. Klein and Croft<sup>10</sup> also reported the expansion coefficients of  $\text{LaF}_3$  from 111–299° K using X-ray method. The data on the lattice parameters reported by the latter authors are in agreement with those reported by Zalkin and Templeton<sup>3</sup>. A perusal of literature shows that there is no data available on the precision lattice parameters and coefficients of thermal expansion of  $\text{CeF}_3$  and other crystals of this type at different temperatures. A programme has therefore been drawn in this laboratory to undertake a complete X-ray study of these crystals with a view to obtaining detailed information on the temperature variation of the lattice parameters, the coefficients of thermal expansion, the positions and thermal parameters of atoms in the unit cell and the Debye temperatures. The present note gives the results of the work done on the lattice thermal expansion of  $\text{CeF}_3$  in the high temperature range.

The powder sample of  $\text{CeF}_3$  used in this investigation was kindly supplied by Dr. L. H. Pierce of Florida State University. The X-ray powder photographs at eight different temperatures were taken employing a high temperature symmetrical back reflection focusing camera and  $\text{CuK}$  radiation. The details of the experimental techniques were described earlier by Suryanarayana<sup>11</sup>. Unambiguous reflections recorded in the Bragg angle region between 69° and 83° were used to evaluate the lattice parameters employing Cohen's<sup>12</sup> least

squares method in combination with an error function  $q \tan q$ . Independent measurements and calculations were made on each film and the average values obtained therefrom are given in Table I.

TABLE I  
Values of the lattice parameters of  $\text{CeF}_3$  at different temperatures

| Temp.<br>in °C | <i>a</i> in Å | <i>c</i> in Å |
|----------------|---------------|---------------|
| 31             | 7.1306        | 7.2805        |
| 74             | 7.1339        | 7.2879        |
| 115            | 7.1380        | 7.2911        |
| 171            | 7.1436        | 7.2971        |
| 212            | 7.1483        | 7.3034        |
| 267            | 7.1551        | 7.3084        |
| 310            | 7.1593        | 7.3115        |
| 340            | 7.1646        | 7.3162        |

The errors in the values of the parameters as calculated by the method of Jette and Foote<sup>13</sup> are  $\pm 0.0005$  Å and  $\pm 0.001$  Å in '*a*' and '*c*' respectively. The values of the two principal coefficients of expansion  $\alpha_a$  and  $\alpha_c$  at different temperatures were evaluated from the temperature/parameter plots by the method suggested by Deshpande and Mudholkar<sup>14</sup> and the following expressions were obtained for their temperature variations:

$$\alpha_a = 11.77 \times 10^{-6} + 76.12 \times 10^{-10} t + 54.10 \times 10^{-12} t^2$$

$$\alpha_c = 13.35 \times 10^{-6} + 85.14 \times 10^{-10} t + 6.87 \times 10^{-12} t^2$$

where *t* is the temperature in °C. The results are shown in Fig. 1.

The values of the two coefficients at 30° C are  $\alpha_a = 12.05 \times 10^{-6}/^\circ\text{C}$  and  $\alpha_c = 13.61 \times 10^{-6}/^\circ\text{C}$ . The mean coefficients of expansion over the range 30–350° C were found to be  $\bar{\alpha}_a = 16.55 \times 10^{-6}/^\circ\text{C}$  and  $\bar{\alpha}_c = 15.39 \times 10^{-6}/^\circ\text{C}$ . The results on the thermal expansion of  $\text{CeF}_3$  at room temperature are compared in Table II with those obtained on  $\text{LaF}_3$  by other investigators.

Though there are differences among the reported values of the coefficients of expansion of  $\text{LaF}_3$ , they agree in regard to the anisotropy, i.e.,  $\alpha_a > \alpha_c$  at room temperature. The present results on  $\text{CeF}_3$  show the opposite, that is, the value of  $\alpha_a$  at room temperature is less than that in the perpendicular direction. However, our

results on  $\text{CeF}_3$  agree with those of Sher *et al.* on  $\text{LaF}_3$ ; in that, the rate of temperature variation of  $\alpha_c$  is less than that along the basal plane. The present results also show that the values of the two principal coefficients of expansion are equal around  $200^\circ\text{C}$ , and above this temperature the value of  $\alpha_a$  is greater than that of  $\alpha_c$ . Since there is no crystallographic transition in  $\text{CeF}_3$ , the observed anisotropy in expansion might be only due to the interplay of different ionic interactions that are present in this crystal. However, a detailed discussion on the similarities and differences in the behaviour of the expansion characteristics of these substances *vis-a-vis* the structure would be incomplete till such data on other isotypic compounds  $\text{PrF}_3$  and  $\text{NdF}_3$  become available. This work has been undertaken by the authors.

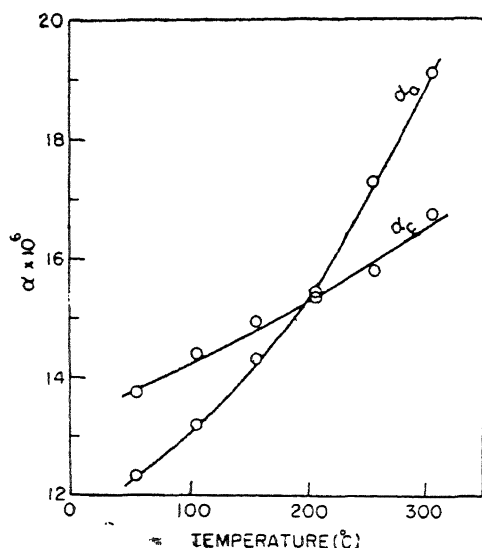


FIG. 1. Temperature variation of  $\alpha_a$  and  $\alpha_c$  of  $\text{CeF}_3$ .

TABLE II

Comparison of the values of the coefficients of thermal expansion of  $\text{LaF}_3$  and  $\text{CeF}_3$  at room temperature

| Substance      | $\alpha_a \times 10^6$ | $\alpha_c \times 10^6$ | Reference                       |
|----------------|------------------------|------------------------|---------------------------------|
| $\text{LaF}_3$ | 15.8                   | 11.0                   | Sher <i>et al.</i> <sup>9</sup> |
|                | 20.0                   | 10.0                   | Klein and Croft <sup>10</sup>   |
| $\text{CeF}_3$ | 12.1                   | 13.6                   | Present study                   |

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## SMALL ANGLE GRAIN BOUNDARIES IN HEULANDITE CRYSTALS

THE study of the configuration of grain boundaries is important to get additional information about the growth of a crystal. Burgers<sup>1</sup>, Bragg<sup>2</sup> and Vogel *et al.*<sup>3</sup> have shown that low angle tilt boundaries are formed by array of edge dislocations. Amelinckx<sup>4</sup> investigated the geometry of dislocation nets and grain boundaries for different crystallographic structures. In the case of small angle grain boundaries it can be shown that  $n = n_b + n_c$ , where  $n_a$ ,  $n_b$ ,  $n_c$  are the number of dislocations per microns in the three branches. Recently Loiacono *et al.*<sup>5</sup> have confirmed this in the case of synthetic lead molybdate crystals—Here we are reporting some of the results obtained from the study of grain boundaries in heulandite crystals. Heulandite belongs to Zeolite family of minerals and is a hydrous calcium aluminium silicate. The chemical formula of heulandite is  $\text{Ca}(\text{AlSi}_7\text{O}_{18}) \cdot 6\text{H}_2\text{O}$ . Heulandite is monoclinic and has a very good cleavage along (010) plane. It is found in the mountain regions

of Switzerland, Italy, U.S.A. and Australia. In India it occurs near Poona.

Etching of the (010) cleavage faces of heulandite crystals in ammonium bifluoride solution revealed closely spaced etch pits arranged in rows. Figure 1



FIG. 1. Y-shaped grain angle boundaries on (010) cleavage of heulandite,  $\times 200$ .

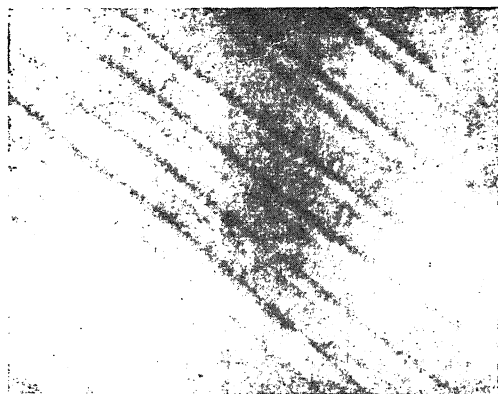


FIG. 2. Polygonized region on (010) cleavage of heulandite,  $\times 200$ .

is a photomicrograph of such a row of pits observed on thin flakes when etched in a solution of 2 gm ammonium bifluoride in 10 cc water. Using a high power objective, individual pits in rows could be clearly resolved. The uniformly spaced pits obtained suggest that they are formed due to low angle grain boundaries separating two grains. The average spacing between pits is measured and the angle of tilt calculated. It is about 36 sec. The pit densities of boundary for intersecting boundaries are noted and at the junction the relation  $n_a = n_b + n_c$  holds good. The results are listed in Table I.

TABLE I

| Number of pits per micron | Branch |       |       |
|---------------------------|--------|-------|-------|
|                           | $n_a$  | $n_b$ | $n_c$ |
|                           | 3      | 1.31  | 1.69  |

The stresses and strains in natural crystals may sometimes produce tilt boundaries due to polygonization. When heulandite cleavage surface is etched in sodium hydroxide solution for one hour at a temperature of about 200° C parallel rows of pits are obtained as shown in Fig. 2. The polygonized region might have formed due to the stresses produced during the process of its growth in nature.

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#### THE KINETICS OF ANODIC OXIDATION OF ZIRCONIUM IN 40% $H_2SO_4$ , AND THE EFFECT OF TEMPERATURE

The kinetics of formation of anodic films on zirconium have been reviewed by Young<sup>1</sup>. In recent times Shatalov and Bondareva<sup>2</sup>, and Bacarella and Sutton<sup>3</sup> have made valuable contributions. Recently we reported on the kinetics of anodic oxidation of zirconium in 0.1 M KOH, 0.05 M  $H_2SO_4$ <sup>4</sup> and 0.1 M KOH + 0.0005 M  $Na_2SO_4$ <sup>5</sup>, and the effect of anion impurities from the radio-tracer studies.

In the present work an attempt is made to study the effect of temperature on the kinetics of anodic oxidation of Zr in 40%  $H_2SO_4$ .

**Experimental.**—Chemically polished zirconium samples were used and the procedure followed is described already<sup>4</sup>.

Closed cells of 200 ml capacity were used. The platinum mesh cathode had 30 cm<sup>2</sup> superficial area to make the double layer capacitance as large as possible. Specimens were suspended vertically from the heavily anodized tag, the minimum amount of which was immersed into the electrolyte. The relative location of the two electrodes was not very critical. The electrolyte used was 40%  $H_2SO_4$  and

all the experiments were carried out at a constant current density of  $4.1 \text{ mA/cm}^2$ .

A Wayne Kerr transformer ratio-arm bridge (B 221A) at 1592 Hz was used to measure the capacitance and the thicknesses of dielectric were calculated assuming the oxide formed a parallel plate capacitor with a dielectric constant of 24.71.

**Results and Discussion.**—The field strength required to maintain a constant ionic current through the film is independent of the thickness of the film<sup>1</sup>. In the present work, anodization was carried out in 40%  $\text{H}_2\text{SO}_4$  at various temperatures ranging from 0° to 75° C. The field across the oxide was  $6.1 \pm 0.1 \times 10^6 \text{ V/cm}$ , independent of thickness and was also found to be approximately independent of temperature, whereas the ionic current density decreased with increase in temperature. The breakdown voltage was found to increase with the increase in temperature (Table I).

TABLE I

Anodic films formed in 40%  $\text{H}_2\text{SO}_4$  at various temperatures

| Temperature<br>°K | Ionic<br>current<br>density,<br>$\text{mA cm}^{-2}$ | Field<br>strength $10^6$<br>$\text{V cm}^{-1}$ | Breakdown<br>voltage, V |
|-------------------|---|--|-------------------------|
| 273               | 4.16  | 5.98   | 80                      |
| 296               | 3.313   | 6.11   | 88                      |
| 314               | 2.729   | 6.11   | 98                      |
| 325               | 2.308   | 6.11   | 114                     |
| 338               | 2.047   | 6.20   | 120                     |
| 348               | 1.495   | 6.00   | 124                     |

The plots of voltage vs time and reciprocal capacitance vs time are linear at a given temperature, showing that the ionic current density was constant.

Guntherschultze and his coworkers<sup>6</sup> were the first to empirically establish, under steady state and high field conditions, that the ionic current density ( $i_i$ ) depends, to a first approximation, on the field ( $F$ ) in an exponential way, viz.:

$$i_i = A_i \exp B_i F \quad (1)$$

$B_i$  is an inverse Tafel constant given by

$$B_i = qa/kT \quad \text{and}$$

$$F = V/D$$

$$\text{or } i_i = A_i \exp qaV/kDT \quad (2)$$

Taking logarithms

$$\text{Log } i_i = \text{Log } A_i + qa/2.303 k \times V/DT \quad (3)$$

When  $\log i_i$  is plotted vs.  $V/DT$  a straight line is obtained (Fig. 1). From the slope, the value of 'a' is found to be  $1.04 \text{ \AA}$  taking the value of  $2e$  for  $q$ . This is because of the anion mobility being predominant for oxide growth on  $\text{Zr}^{7-8}$ . The value of 'a' deduced is comparable to the mean separation of the oxygen ions in  $\text{ZrO}_2$  ( $1.66 \text{ \AA}$ ).

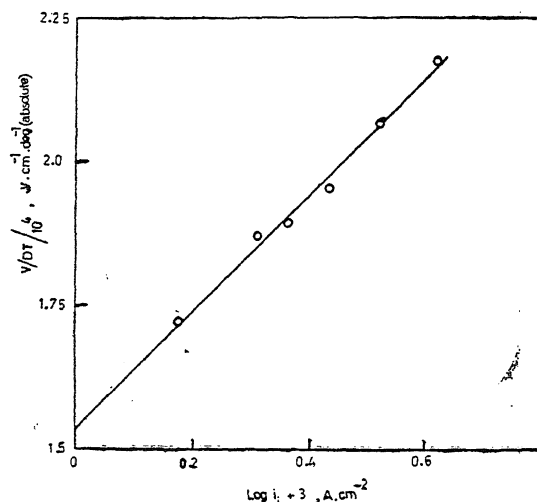


Fig. 1.  $\text{Log } i_i$  vs  $V/DT$  for films formed in 40%  $\text{H}_2\text{SO}_4$  at a constant current density of  $4.1 \text{ mA/cm}^2$ .

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SYSTEMATICS OF E2 TRANSITIONS FROM  
0<sup>+</sup> TO 2<sup>+</sup> STATE IN SOME ACTINIDES

THE comparison of experimentally determined gamma-ray transition probabilities between nuclear states with theoretical predictions is one of the most fruitful methods for testing nuclear models. E2 transitions are of wide occurrence throughout the periodic table and they are famous for large deviations (10 to 400 times) from single particle estimates which suggest a sort of collective movement of the nucleons inside the nucleus. To calculate E2 transition probabilities from 0<sup>+</sup> ground state to 2<sup>+</sup> first excited state in the actinide region treating the unit radius as free parameter within the Davydov and Rostovsky<sup>1</sup> (DR) formalism is of present interest.

$\epsilon_{20}$ ,  $\epsilon_{22}$  and  $\epsilon_{0\beta}$  are the energies of first 2<sup>-</sup>, second 2<sup>+</sup> and first excited 0<sup>+</sup> states respectively and hence S and q are calculated from experimental energy levels. So our calculations are for those nuclei for which experimental data are available. The deformation parameters  $\beta_0$ 's are taken from literature. We have treated the nuclear unit radius  $\gamma_0$  as free parameter and determined by arithmetic mean of unit radii obtained from experimental data using the formula (1). It is interesting to note that the value of  $\gamma_0$  (= 1.27 fm) we obtained in the actinide region is equal to that employed by Blomqvist and Wahlborn<sup>2</sup> to calculate single particle energies and wave functions in the lead region. Our B(E2; 0<sup>+</sup> → 2<sup>+</sup>) values together with single particle estimates are given in Table I.

TABLE I

| Nucleus           | $E_\gamma$<br>(0 <sup>+</sup> → 2 <sup>+</sup> )<br>(KeV) | $\beta_0$ | S     | q     | B(E <sub>2</sub> ; 0 <sup>+</sup> → 2 <sup>+</sup> )<br>DR<br>(e <sup>2</sup> · 10 <sup>-48</sup><br>cm <sup>4</sup> ) | B(E <sub>2</sub> ; 0 <sup>+</sup> → 2 <sup>+</sup> )<br>Exp<br>(e <sup>2</sup> · 10 <sup>-48</sup><br>cm <sup>4</sup> ) | F <sub>DR</sub> | F <sub>SP</sub> | $\gamma_0$ |
|-------------------|---|-----------|-------|-------|--|---|-----------------|-----------------|------------|
| <sup>228</sup> Th | 57.8  | .225      | 15.3  | 14.4  | 7.15   | 7.12 ± .75  | 0.996 ± .105    | 172 ± 18        | 10.1       |
| <sup>230</sup> Th | 53.0  | .233      | 13.0  | 14.5  | 7.79   | 7.9 ± .8  | 1.015 ± .103    | 189 ± 19        | 10.6       |
| <sup>232</sup> Th | 50.0  | .251      | 15.82 | 14.56 | 9.11   | 9.7 ± .5  | 1.064 ± .055    | 229 ± 12        | 9.6        |
| <sup>232</sup> U  | 47.0  | .257      | 14.56 | 18.21 | 10.38  | 9.9 ± 1.2   | 0.954 ± .116    | 234 ± 28        | 9.2        |
| <sup>234</sup> U  | 43.5  | .251      | 21.2  | 18.62 | 9.99   | 10.0 ± .8   | 1.001 ± .080    | 234 ± 19        | 8.6        |
| <sup>238</sup> U  | 45.3  | .262      | 21.04 | 24.26 | 11.40  | 11.5 ± 1.4  | 1.001 ± .126    | 266 ± 32        | 8.7        |
| <sup>238</sup> U  | 44.7  | .281      | 23.76 | 22.24 | 13.11  | 12.6 ± 0.6  | 0.961 ± .046    | 288 ± 14        | 8.3        |
| <sup>238</sup> Pu | 44.1  | .271      | 21.38 | 23.35 | 12.81  | 12.63 ± 0.17  | 0.986 ± .013    | 288 ± 4         | 7.9        |
| <sup>240</sup> Pu | 42.9  | .278      | 20.11 | 22.03 | 13.55  | 12.7 ± 0.4  | 0.937 ± .030    | 287 ± 9         | 8.2        |

In this model the collectivity of E2 transitions is accounted in terms of the interactions of  $\beta$  and  $\gamma$ -vibrations and the reduced E2 transition probabilities inside the ground rotational band are given as:

$$B(E2; J_i 0 \rightarrow J_f 0) = \frac{e^2 Q_0^2}{16\pi} 5(2J_i 00 J_f 0)^2 \left(1 - \frac{1}{S}\right) \left(1 - \frac{2}{3} \frac{S}{q^2}\right)^2 \quad (1)$$

$$Q_0 = \frac{3ZR_0^2\beta_0}{\sqrt{5\pi}}$$

where

$$S = \frac{\epsilon_{22}}{\epsilon_{20}}$$

$$q = \frac{\epsilon_{0\beta}}{\epsilon_{20}}$$

$$R_0 = \gamma_0 A^{1/3}, \quad \gamma_0 = 1.27 \cdot 10^{-13} \text{ cm}$$

Table I shows that the calculated B(E2, 0<sup>+</sup> → 2<sup>+</sup>) values are quite close to the experimental data<sup>3</sup>. The factor F<sub>DR</sub> ranges between 0.95 to 1.06 whereas the factor F<sub>SP</sub> varies between 170 to 290 which indicates the superiority of DR estimates in predicting the transition probabilities between various collective states.

$$F_{DR} = \frac{B(E2)_{exp}}{B(E2)_{DR}}$$

$$F_{SP} = \frac{B(E2)_{exp}}{B(E2)_{SP}} \quad (\text{E2 enhancement factor})$$

B(E2; 0<sup>+</sup> → 2<sup>+</sup>) are also calculated in rare-earth region by Abecasis *et al.*<sup>4</sup>. But for some nuclei, where the deviations are more than 50%, the agreement with experimental data is within 20% in the rare-earth region whereas among actinides the present agreement is within 5%. The deviations,

however, can be brought down to 10% among rare-earths by increasing  $\gamma_0$  to 1.32 fm which is also obtained by treating  $\gamma_0$  as free parameter. This value of  $\gamma_0$  was employed by Pery and Buck<sup>5</sup> in optical model calculations.

From Fig. 1 drawn between non-axiality parameter  $\gamma_0$  and enhancement factor  $F_{SP}$ , it is observed

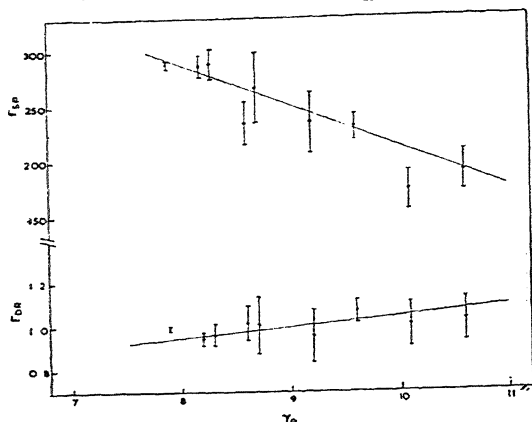


FIG. 1. Variations of the factors  $F_{SP}$  and  $F_{DR}$  with the non-axiality parameter  $\gamma_0$ .

that  $F_{SP}$  shows a decreasing trend with increase of  $\gamma_0$  whereas  $F_{DR}$  shows increasing tendency with increase of  $\gamma_0$  as shown earlier by Rajput<sup>6</sup>, in this actinide region also.

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### POTENTIOMETRIC STUDY OF THE COMPLEXATION REACTION OF ALUMINIUM(III) WITH *m*-HYDROXY BENZOIC ACID AND *p*-HYDROXY BENZOIC ACID

THE complexation reaction of aluminium with a number of hydroxy acids is known. In a previous communication, complexation reaction of aluminium with salicylic and sulfosalicylic acid has been reported<sup>1</sup>. The present work reports the complexing property of aluminium (III) with *m*- and

*p*-hydroxy benzoic acids. The Bjerrum-Calvin titration technique as used by Irving and Rossotti<sup>2,3</sup> is used for the calculation of proton-ligand and metal-ligand stability constant.

The experimental is as reported earlier<sup>1</sup>.

The calculation of "practical" proton-ligand stability constant was carried out by plotting a group of  $\bar{n}_A$  against pH and applying the method of interpolation at half  $\bar{n}_A$  values and interpolation at various  $\bar{n}_A$  values. The values, with error limit  $\pm 0.03$  are given in Table I.

TABLE I  
Values of the protonation constants of the ligands  
Temp. = 27° C

| Acid                           | Constant       | $\mu=0.10$ | 0.20  | 0.30  | 0.40  |
|--------------------------------|----------------|------------|-------|-------|-------|
| <i>m</i> -hydroxy benzoic acid | $\log K_1^H$   | 10.63      | 10.55 | 10.47 | 10.42 |
|                                | $\log K_2^H$   | 4.28       | 4.25  | 4.21  | 4.18  |
|                                | $\log \beta^H$ | 14.91      | 14.80 | 14.68 | 14.60 |
|                                | $\log K_1^H$   | 9.17       | 9.10  | 9.03  | 8.98  |
| <i>p</i> -hydroxy benzoic acid | $\log K_2^H$   | 4.19       | 4.15  | 4.12  | 4.09  |
|                                | $\log \beta^H$ | 13.36      | 13.25 | 13.15 | 13.07 |

TABLE II  
Values of concentration stability constant of complexes  
Temp. = 27° C

| System                         | Constants    | $\mu=0.10$ | 0.20  | 0.30  | 0.40  |
|--------------------------------|--------------|------------|-------|-------|-------|
| Al (III)-                      | $\log K_1$   | 8.98       | 8.87  | 8.79  | 8.70  |
| <i>m</i> -hydroxy benzoic acid | $\log K_2$   | 8.23       | 8.19  | 8.15  | 8.11  |
|                                | $\log \beta$ | 17.21      | 17.06 | 16.94 | 16.81 |
| Al (III)-                      | $\log K_1$   | 7.83       | 7.75  | 7.67  | 7.61  |
| <i>p</i> -hydroxy benzoic acid | $\log K_2$   | 6.98       | 6.92  | 6.87  | 6.82  |
|                                | $\log \beta$ | 14.81      | 14.67 | 14.54 | 14.43 |

The metal ligand stability constants were calculated by analysis of the formation curves obtained by plotting  $\eta$  against pL. Various computational methods<sup>4</sup> were applied to evaluate stepwise formation constants. The error limits are  $\pm 0.03$  for  $\log K_1$  and  $\pm 0.05$  for  $\log K_2$ . Precipitation was observed at pH 5.6 and 5.8 for

*m*- and *p*-acid respectively. Only lower pH regions were used for calculations to avoid error due to hydrolysis. The mean values of the concentration stability constants are given in Table II.

Thermodynamic formation constants were obtained by extrapolation of the measured formation constants to zero ionic strength. The value obtained for Al(III)-*m*-hydroxy benzoic acid system is 17.35 and for Al(III)-*p*-hydroxy benzoic acid system is 14.93.

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#### MOLECULAR ORBITAL CALCULATIONS ON THE DONOR INTERACTION OF HALIDE IONS

SINCE the completion of the studies on the donor interaction of halide ions<sup>1</sup>, there has been recent evidence for the formation of stable complexes of halide ions with carbon tetrahalides<sup>2</sup>. It was considered interesting to carry out molecular orbital calculations on halide ion-carbon tetrahalide ( $X^-CX_4$ ) systems. The interaction of  $F^-$  and  $Cl^-$  with  $CF_4$  and  $CCl_4$  was examined by employing the extended Huckel calculations<sup>3,4</sup>. Two orientations of halide ions with respect to  $CX_4$  molecules were considered: I, where  $X^-$  is in the plane of a  $CX_2$  unit along the bisector of the  $XCX$  angle and II, where the  $X^-$  is along the  $C_{3v}$  axis of the  $CX_3$  unit. The results are summarised in Table I.

TABLE I

| $X^-$  | Acceptor | Orientation | $X^- \cdots C$ (Å) | $D_e$ , kcal     |
|--------|----------|-------------|--------------------|------------------|
| $F^-$  | $CF_4$   | I           | 4.25               | 0.002            |
|        |          | II          | 3.00               | 0.044            |
|        | $CCl_4$  | I and II    | ..                 | no stabilization |
| $Cl^-$ | $CF_4$   | I           | 2.9                | 5.90             |
|        |          | II          | 3.7                | 0.03             |
|        | $CCl_4$  | I           | ..                 | no stabilization |
|        |          | II          | 3.8                | 0.04             |

It is evident that there is some stabilization in some of the cases, the maximum stabilization being in the  $Cl^-CF_4$  system (orientation I). The only experimental  $D_e$  values available in the literature<sup>1</sup> are  $\sim 2.5 \pm 1$  kcal mole<sup>-1</sup> for the interaction of  $I^-$  and  $Br^-$  with  $CCl_4$  in  $CH_2Cl_2$  solution. The experimentally observed donor ability of halide ions towards carbon tetrahalides<sup>2</sup> (with the exception of  $CF_4$  which has not been used as acceptor) varies in the order,  $I^- > Br^- > Cl^-$ , as expected on the basis of the ionization potentials. Based on this order of donor ability,  $F^-$  should be the weakest donor among halide ions. Also, the association constant for the  $Cl^-CCl_4$  system could not be experimentally determined<sup>1</sup>. The magnitude of association between  $Cl^-$  and  $CCl_4$ , if any, appears to be very small. These results are in accord with the calculations reported here.

It appears that  $CF_4$  is a better electron acceptor than  $CCl_4$ . The difference in stability of the  $Cl^-CF_4$  complex with a change in configuration may be attributed to the change in  $Cl^-F$  distance. The lesser  $Cl^-F$  distance in orientation I will make the release of electron from  $Cl^-$  more facile than in the case of orientation II.

The author is thankful to Professor C. N. R. Rao of the Indian Institute of Technology, Kanpur, for suggesting the problem and guidance.

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#### ATTEMPTS ON THE CHEMICAL TRANSFER OF MEMORY FROM TRAINED TO UNTRAINED KITTENS

DURING the last decade an enormous amount of work has been done to transfer memory from trained animals to the untrained ones<sup>1-5</sup> both in mice and goldfish. The results that have been reported present a conflicting and confusing picture<sup>6</sup>; trained extracts have been variously reported as loaded with<sup>7</sup>, or depleted of<sup>8</sup> suitable material after training on various tasks. As a part of an investigation on the biochemistry of memory, we tested the effect of the extracts from trained kittens on the untrained ones. Our investigation eliminates many of the objections raised on the earlier experiments<sup>6</sup>, and the text below discusses it in detail.



**Materials and Methods.**—We had kittens grown in darkness from birth; they were exposed to the horizontal patterned environment in an experimental set up very similar to that used by Blakemore and Cooper<sup>9</sup>. The kittens (I) were exposed to this stimuli for 20 days (3 hr a day) from three weeks of age, when their brain is most susceptible to changes in the environment<sup>10-11</sup>. After this training their brains were removed for the extraction of the 'chemical substance' involved. Simultaneously another group of kittens was exposed to a non-patterned stimuli; they (II) underwent identical training procedure, except for the stimuli, and their brains were also removed after the 20-day training. A third group of kitten (III), grown

pooled together. The low-molecular weight fraction (fraction after dialysis), which is responsible for the chemical transfer of memory, was obtained following the procedure of Ungar, *et al.*<sup>12</sup>.

The receiver group of kittens consisted of animals aged about 28 days, and grown till then in total darkness from birth. The extract, obtained from the above procedure, was administered intraperitoneally to the naive kittens. The amount injected was 1 g/kitten. The three groups of kittens receiving extracts from the I, II, and the III groups of donor kittens were respectively called TER (trained extract receiver group), NER (naive extract receiver group) and DTER (dark trained extract receiver group).

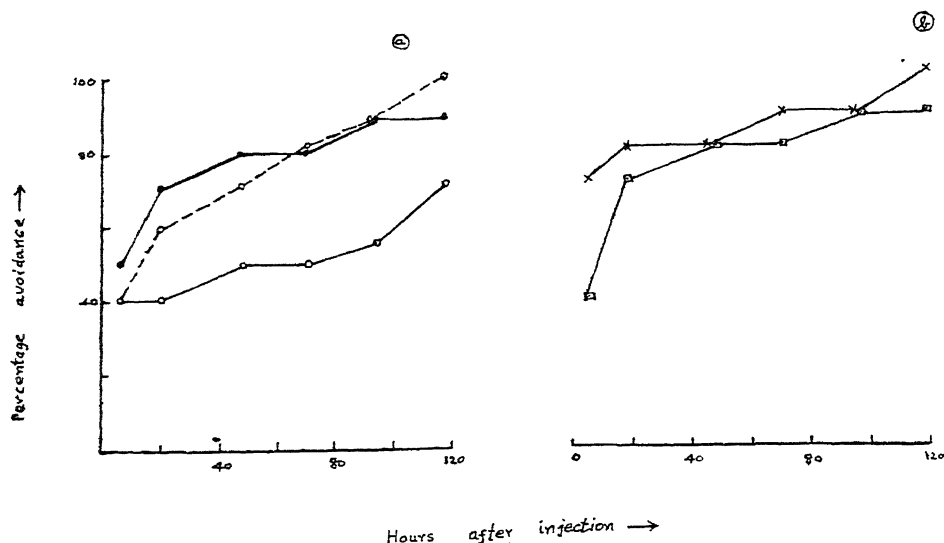


FIG. 1. Information transfer curves: Curves in (a) summarise the effect on the behaviour of different groups of kittens by the injection of various extracts: each is the response curve after the administration of the extract; for the TER group  $\bigcirc-\bigcirc-\bigcirc$ ; for the NER group  $\bullet-\bullet-\bullet$ ; and for the DTER group  $\bigcirc---\bigcirc---\bigcirc$ . (b) these are similar curves for a normally grown kitten (N)  $\times-\times-\times$ ; and for the DR group  $\square-\square-\square$ . Abscissae, interval between injection of extract and testing (hr); and ordinate, percentage avoidance in the testing box.

in complete darkness, was also sacrificed for extraction. The kittens from groups II and III served as active controls. Each of these donor groups consisted of six kittens.

Prior to dissection of the kittens, their response to a vertically oriented stick (moved) was noted. Group I kittens did not show any response, kittens from the second group actively responded by putting its paw out, and the last group of kittens reluctantly and passively attempted to hold the stick occasionally. This is in accordance with the expected results of the environmental modification (Blakemore, C., personal communication). The sacrificed brains from kittens similarly trained were

The extent of memory transfer in these different receiver groups were found by the behaviour of these kittens in a long box containing 10 vertical bars placed at different distances at random, when allowed to walk through it; the better it sees the vertical orientation the more bars will it be able to avoid. Thus the number of collisions these animals make directly gives the extent of transfer. The data are presented in Fig. 1 a. For the sake of comparison the behaviour of a cat grown in normal environment (N) and of another grown in total darkness except during the test period (DR) are also given (Fig. 1 b). These kittens were also of the same age as the receiver kittens.

**Results and Discussion.**—All kittens in the three different receiver groups behaved in a very similar manner initially (comparing the initial score of the DR kitten and the other receiver kittens). But during the course of the later tests the difference became more vivid. Generally there is increase in the scores of all kittens with time excepting the TER group of kittens; there was an increase in the number of bars avoided with further testing. 12–48 hr after the injection of the extract, the TER group showed 'non-recognizability, to the same degree as before, while the increase in avoidance was marked among other groups. The most remarkable difference was during the next 48 hr when the TER group scores remained virtually at the same level while other kittens in other groups showed complete recognition. Even at 120 hr after the injection of the extract, when all the groups were perfectly normal, the TER group of kittens were still 'blind' to 'a certain level'.

The difference in scores of the TER group and the NER, N and DR groups is statistically significant ( $p < 0.001$ ); a comparison of the TER group with the DTER group also shows that the differences are significant ( $p < 0.01$ ). It must however be mentioned that the intensity of behaviour of the donor kittens is much more than the receiver kittens, when tested in the testing box.

From what is said above, the following conclusions can be drawn: the transfer of orientation specific memory has occurred, i.e., a preference for the horizontal orientation. This specificity is very clear in the receiver group (TER) although was weaker than the donor kittens. Unless the memory is repowered, the specificity for the horizontal orientation becomes poor, and almost no effect after 6–8 days in the TER group. This may be attributed to one of the following reasons: the still prolonged critical period, or to the limited action of these extracts on behaviour or to the plasticity of the brain which resists the influenced behaviour or to habituation to other orientations.

Our experiments are ideal since they do not involve any stress on the animal. Also the experimental task is only an unusual environment and does not involve a totally different task, whose output cannot be strictly equated to learning. According to the results presented, the chemical transfer of memory is probable under the above test conditions. Further attempts to confirm these results with the exact chemical involved in the memory transfer and cross experimentation are underway and will be discussed in detail elsewhere.

We do not believe these molecules are the memory molecules, which act as the ultimate store house of

the information received. It is more probable that the arrangements of the neurones and their interconnections are the ultimate source of memory<sup>10-14</sup>. But the formation of such interconnections may need some chemical mediators, and these may be the ones that are extracted and are involved in memory transfer. Thus these could play a similar role in receiver brains as well. Thus overtraining and delay in extraction may decrease the quantity of these mediators, and thus affecting the extent of transfer.

The exact nature of learning and memory formation is still at its infant stage, and requires more evidences before definite conclusions can be drawn.

The authors express their gratitude to Prof. G. Ungar and Dr. C. Blakemore for their advice and comments. We are also thankful to Professors I. M. Mathai and P. J. Sanjeevaraj for help and guidance.

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ON A NEW INTERSTITIAL SPECIES OF  
*TROCHODOTA* (APODIDA, HOLOTHURIODEA),  
FROM ANDAMANS, INDIA

IN the course of an investigation of interstitial fauna of Andaman Islands during April-May 1973, four specimens of a tiny holothurian, belonging to the genus *Trochodota* Luda<sup>1</sup>, were collected in the intertidal sands of Havelock Island. The specimens differ from all the known species of the genus and hence, they are described here as a new species under the name *Trochodota havelockensis*.

**Description.**—The holothurians measured 2.5–3.4 mm in length, depending on the state of contraction and about 0.4–0.5 mm in maximum diameter. Body cylindrical and vermiform, with anterior end slightly swollen. Cuticle rough. Integument transparent with a pinkish tinge and viscera can be seen through it. Mouth and anus terminal. Oral disc fringed with a circle of 10 tentacles equal in length and joined together at base by buccal membrane. Tentacles distally bifurcated and attain a length of 0.3–0.4 mm depending on the state of contraction; they can partly be retracted into mouth.

Dermis contains four types of calcareous deposits, viz. sigmoid bodies, C-shaped spicules, wheels and supporting rods. First three categories of ossicles loosely scattered throughout body wall, while supporting rods alone occur in connective tissue of tentacles. Sigmoid bodies and C-shaped spicules 48–62  $\mu$  wide; both deposits smooth, with their ends tapered and curved in same plane. Wheels slightly hexagonal and 38–48  $\mu$  in diameter. Each wheel has 6 spokes, with smooth rim on outer and inner margins. All wheels are of same structure and their developmental stages not seen. Supporting rods smooth, 40–56  $\mu$  long, slightly curved with tapering ends and their long axes mostly parallel to that of tentacle or digit in which they lie. Four stages of rods seen with their size and branched ends. Calcareous ring consists of 10 similar, slender, slightly biconcave and contiguous pieces; plates alternate with five pairs of tentacles and statocysts. Radials not pierced for passage of nerves. Two tentacles occur in each interradius. Cartilaginous ring wanting.

Polian vesicle elongated and 70  $\times$  20  $\mu$  in size. Single stone canal ends blindly in body cavity. Alimentary canal straight, with spacious stomach and long intestine. Five pairs of oval statocysts about 40  $\times$  25  $\mu$  in size occur in a circle on tentacular collar; each encloses a solitary statolith. Eye spots wanting. Sexes separate. Gonads consist of two small bunches with short gonoducts. Method of reproduction not known.

**HABITAT.**—Adult specimen 2.8 mm long with genitalia collected by the author on 8-5-1973. Deposited in Zoological Survey of India, Catalogue Rept. No. E 453 1.

**LOCALITY.**—Intertidal zone, Havelock Island, Lat. 12° 44' 10" N and Long. 92° 59' 20" E), Andamans, India.

**Remarks.**—Of the ten species of *Trochodota* hitherto known, *T. havelockensis* is closely related to *T. turpanilla* Salvini-Plawen<sup>2</sup> (the only earlier known interstitial species of the genus) in general organization of body, particularly the digits of tentacles and calcareous deposits of skin. However, the new species is clearly distinguished from the latter by the detailed structure of dermal ossicles, such as smoothness of supporting rods, curvature of C-shaped spicules in same plane, wheels with smooth margins, etc.

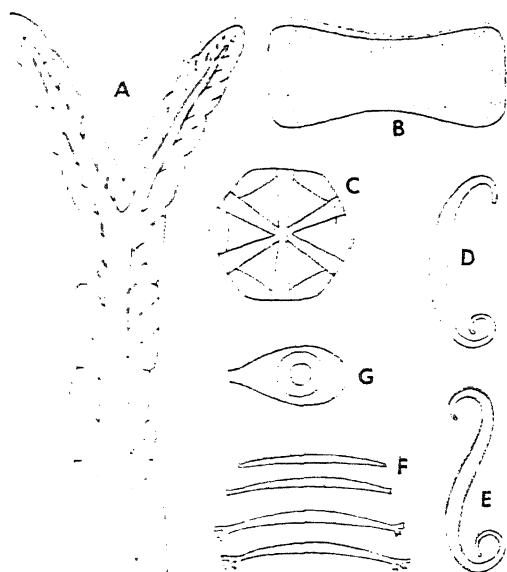


FIG. 1. *Trochodota havelockensis* n. sp. A, Tentacle; B, Piece of calcareous ring; C, Wheel; D, C-Shaped spicule; E, Sigmoid spicule; F, Supporting rods; G, Statocyst.

Until now, *Psammothuria ganapati* Rao<sup>3</sup> is the only interstitial holothurian recorded and described in Indian waters. Thus, *T. havelockensis* is the second interstitial holothurian recorded on Indian coast while representing first record for the Andaman Islands. The discovery of *T. havelockensis* is of considerable importance in extending geographical distribution of the genus to Bay of Bengal thus bridging our knowledge of the fauna of Pacific, Atlantic and European waters.

**Ecological notes.**—The holothurians were collected in coarse sands mixed with fine shell gravel and

from 10 to 15 cm depths (10-30 cm below surface between the low and high-tide levels of intertidal zone). The sands are mostly coralline and sub-spherical; their texture ranged between 400 and 700  $\mu$  in mean diameter. Temperature in the habitat varied between 28°C and 29°C, while salinity of interstitial water measured 34.2. The animals are agile and exhibit a positive thigmotaxis, with the power of adhesion mainly confined to tentacles.

Due to non-availability of relevant literature, a brief description and rough drawings of the species were sent to Dr. L. V. Salvini-Plawen, Zoologisches Institut der Universität Wien, Austria. The author is grateful to him for confirming identity of the species as new to science.

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# PHYTOCHROME MEDIATED ROOT REGENERATION IN MUNG (*PHASEOLUS AUREUS* ROXB.) AND MANGO (*MANGIFERA INDICA* L.) CUTTINGS

DURING the course of investigations on mung bean bioassay of rooting cofactors in mango cuttings, it was observed that the rooting in etiolated mung cuttings, taken under incandescent light, was better than those taken under green light. This photo-response of mung cuttings led to a study of the effect of different wavelengths of light on rooting of mung and mango cuttings. These investigations comprised of preliminary studies with mung hypocotyl cuttings and extension of these results to mango cuttings later.

The studies with mung bean (var. Pusa Baisakhi) hypocotyl cuttings were conducted using the technique of Hess<sup>6</sup> with some modifications. Hypocotyl cuttings of 7 cm length were taken under green light from etiolated seedlings and placed in 5 x 2 cm vials, the outer surface of which was darkened and plugged properly with non-absorbant cotton, so that only 2 cm of upper end of the cuttings received light irradiations. Non-leafy, basal cuttings of mango, about 30 cm in length were collected from stool bed and were treated with 5,000 ppm IBA for 15 seconds. The cuttings were planted in pots having equal parts of sphagnum moss, sand and grit mixture. The details of the light treatments given to mung and mango cuttings have been given in Tables I and II. Various lights used in

the irradiations were selected from the spectrum of low intensity along with dark control.

TABLE I  
Effect of quality of light on rooting of mung hypocotyl cuttings

| Treatments*        | Irradiated<br>100 times | Non-irradiated |
|--------------------|-------------------------|----------------|
|                    | No. of roots            | No. of roots   |
| Dark (Control)     | 19.00                   | 15.22          |
| Normal light       | 17.75                   | 19.94          |
| Red light (R)      | 25.88                   | 34.28          |
| Far Red light (FR) | 12.75                   | 14.29          |
| R-FR               | 12.70                   | 14.32          |
| R-FR-R             | 28.55                   | 32.50          |
| R-FR-R-FR          | 13.05                   | 14.75          |
| R-FR-R-FR-R        | 28.35                   | 32.65          |
| * F value          | 45.57*                  | 20.84*         |
| SE <sub>i</sub>    | 0.18                    | 0.25           |
| C.D. 5%            | 0.38                    | 0.57           |

\* Significant at 1% level.

\* The light irradiations were given at 10 hour interval for 5 minutes after taking cuttings. The number of roots were counted on 8th day. In each treatment there were 4 replicates of 10 cuttings each. Analysis of variance was done after square root transformation of original values.

The red light irradiation of mung hypocotyl cuttings gave the maximum number of roots compared to dark control and normal light. In a series of alternate irradiations with red, far red lights, there was significant promotion of roots when red light irradiation was the last. Whenever, the far red light irradiation was in the end, there was significant inhibition of rooting and it was on par with the dark control. Mango cuttings also reacted favourably to red light treatments as seen from Table II. Red light irradiations of either completely etiolated cuttings or those kept in normal light, strongly promoted the percentage of the rooted cuttings as well as the number of roots per rooted cutting (Fig. 1).

Thus, the strong promotive effect of red light in rooting of the cuttings and far red reversal of this effect amply prove that the phytochrome system is operative in the root regeneration of cuttings. Many of the morphogenetic responses of plants to red light and their reversal by far red light have been attributed to phytochrome action ever since phytochrome was discovered<sup>9</sup>. Although many physiological and morphogenetic phenomena have been reported to be under the control of phytochrome, there appears to be no report so far, about the phytochrome mediated rooting. The suggestion that phytochrome mediates rooting should not be misconstrued, as

TABLE II  
Effect of quality of light on regeneration of mango cuttings

| Treatments:                    | Percentage of rooting |               | No. of roots |
|--------------------------------|-----------------------|---------------|--------------|
|                                | Percentage            | Angular value |              |
| 1. Normal light (NL)           | 41.66                 | 40.12         | 4.41         |
| 2. Etiolation - NL             | 33.33                 | 35.24         | 4.25         |
| 3. Etiolation - NL - Red light | 91.66                 | 77.04         | 18.35        |
| 4. Etiolation - Red Light      | 91.66                 | 77.04         | 12.65        |
| 5. Etiolation - Far Red Light  | 41.66                 | 40.12         | 8.85         |
| 6. Continuous dark             | 50.00                 | 45.00         | 4.66         |
| * F value                      |                       | 7.55*         | 50.48†       |
| SE <sub>i</sub>                |                       | 9.94          | 0.18         |
| CD 5%                          |                       | 24.32         | 0.45         |

\* Significant at 5% level.

† Significant at 1% level.

‡ Treatments: 1. Cuttings were kept in normal day light during the daytime. 2. Cuttings were etiolated for 5 days and then transferred to normal light conditions. 3. Cuttings etiolated for 5 days, transferred to normal light were given red light irradiations for 30 minutes at 12 hr interval. 4. Cuttings kept in continuous darkness were given red light irradiations at 12 hr interval. 5. Cuttings kept in dark were given far red light irradiations, at 12 hr interval for 30 minutes. 6. Cuttings kept completely in dark.

an absolute control of rooting by phytochrome, especially as some amount of rooting in mango occurred under total darkness. These results suggest that phytochrome controls production of some factors which, in combination with auxin, enhance the rooting of cuttings. It is well established now<sup>1,4,9,11</sup> that the activity of the enzyme phenylalanine ammonia lyase (PAL) which control the synthesis of phenyl propanoid compounds, flavonoids and anthocyanins is under the control of phytochrome. Equally established is the fact that besides auxins, certain rooting cofactors are essential for regeneration of cuttings of many plants and that these rooting cofactors are phenolic in nature<sup>7,8,10</sup>. Anthocyanins are also closely associated with rooting of cuttings<sup>2</sup>. It is, therefore,

reasonable to assume that phytochrome controls the synthesis of the rooting cofactors which in turn trigger the root initiation in conjunction with auxins.

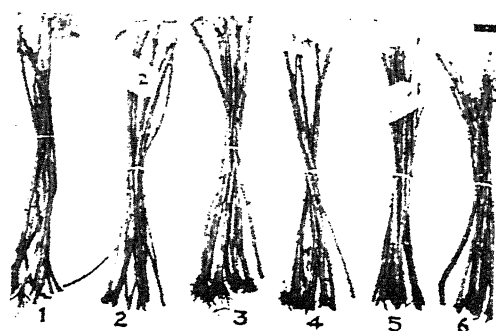


FIG. 1. Effect of quality of light on regeneration of mango cuttings. 1. Cuttings kept in normal day light during daytime. 2. Cuttings were etiolated for 5 days and then transferred to normal light conditions. 3. Cuttings etiolated for 5 days, transferred to normal light, were given red light irradiations for 30 minutes at 12 hr intervals. 4. Cuttings kept in continuous darkness were given red light irradiations at 12 hr interval for 30 minutes. 5. Cuttings kept in dark were given far red light irradiations, at 12 hr interval for 30 minutes. 6. Cuttings kept completely in dark.

The significance of these findings is that mango cuttings—a difficult to root plant material—could be induced to root to a maximum extent through the action of phytochrome system. This holds ample promise that this principle can gainfully be employed in the propagation of other fruit crops through cuttings.

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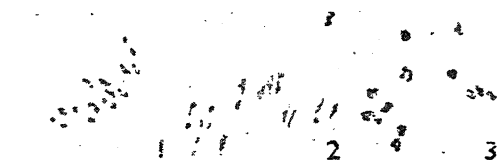
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# CYTOGENETICS OF SEMI-ARID PLANTS

## II. Cytological Studies in *Corallocarpus conocarpus* Dalz & Gibbs. of Cucurbitaceae

GENUS *Corallocarpus* is represented by five species in India<sup>1</sup>. *Corallocarpus conocarpus* is found growing wild as a climber on *Euphorbia cadicifolia* in the hillock around Jodhpur<sup>1</sup>. This species has 3-5 angled, arrow-shaped leathery leaves with minute white hairs on both the surfaces. Male peduncle is shorter than the entire length of leaves. Male flower has three stamens, the female flower is fasciculate and the fruit is a berry. Fruit is rounded at the base and pointed at apex (2.5 x 1.75 cm in size), on ripening they become red. Seeds are ellipsoidal and at maturity, they are black with a velvety surface.

The technique followed is described in an earlier paper<sup>2</sup>. An analysis of dividing pollen mother cells at diakinesis and metaphase I showed regular twelve bivalent formation (Fig. 1) in 25 (out of the 30) cells studied; while two cells showed thirteen bivalents (Fig. 2) and the other three cells fourteen bivalents (Fig. 3). A single nucleolus was commonly attached to one nucleolar organising chromosome in all the three types of the cells studied. At the metaphase I, the range of ring and rod bivalent was 6-9 and 3-6 with mean 9.55 and 2.45 respectively in the cells having twelve bivalents. Further stages of meiosis were regular, culminating into normal tetrad formation.



FIGS. 1-3. Fig. 1. Meiosis in *Corallocarpus conocarpus*—1. Showing 12<sub>n</sub>; 2, showing 13<sub>n</sub> and 3, showing 14<sub>n</sub>.

The base number for this genus is not known and none of the Indian species has so far been worked out cytologically. However the somatic number  $2n = 72$  for *C. welwitschii* was reported from Africa<sup>3</sup>, but without mentioning the base number. The present investigation revealed twelve as the base number for this genus, as indicated by the regular twelve bivalent formation in most of the gametic cells. In the family Cucurbitaceae also the most common base number is twelve. Other numbers  $n = 13$  and  $n = 14$  noted, in a few cells during the present investigation, appeared to be the derived ones. The regular bivalent formation is further indicative of the diploid nature of this species.

The appearance of fourteen and thirteen bivalents in some of the cells are indicative of aneusomy in this species, which is probably the first report in Cucurbitaceae. It was observed in the material collected from 3-4 climbers, to see whether the aneusomy was prevalent in groups or in individual plants. The buds were collected from a single plant, which survived during the rainy season of 1974 and the same aneusomy was noted. In *Chrysanthemum* such variation in chromosome number between the cultivars and the individual plants has been noticed<sup>4</sup>. It has been pointed out that in most of the aneusomatic taxa the chromosome number is approximately equal to their diploid number. Where the increase or decrease involves only a few chromosomes, the mechanical basis involved could be non-disjunction and/or lagging of the chromosomes with their ultimate loss. This has been clearly shown by Dowrick to be operative in aneusomatic taxa of *Chrysanthemum*<sup>5</sup>.

Thanks are due to Prof. H. C. Arya for the facilities and to Dr. S. N. Raina and Dr. M. N. Tewari for help.

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## EFFECT OF SOME NATURALLY OCCURRING PLANT PRODUCTS ON SOUTHERN SUNNHEMP MOSAIC VIRUS (SSMV)

THERE are numerous records<sup>1-4</sup> dealing with natural or synthetic compounds that inhibit infectivity of plant viruses with varying degrees of efficiency. The use of synthetics for controlling plant diseases, however, has to be practised with caution because of the dangers of their adventitious residual effects. As such, a long range programme has been undertaken in this laboratory to study the effect of different classes of natural products for the control of plant virus diseases. The effects of flavonoids and coumarins as inhibitors of infectivity of southern sunn hemp mosaic virus on its local lesion host

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TABLE I  
Inhibition of growth of *S. blight* by various Cytopos  
... ..

| Concentration | Average number of<br>seedlings |         | Inhibition<br>(%) |
|---------------|--------------------------------|---------|-------------------|
|               | Control                        | Treated |                   |
| 1. Kharif     | 154                            | 153     | 0.6               |
| 2. Rabi       | 154                            | 121     | 21.5              |
| 3. Kharif     | 154                            | 137     | 10.1              |
| 4. Rabi       | 154                            | 71      | 52.4              |
| 5. Kharif     | 154                            | 114     | 21.5              |
| 6. Rabi       | 154                            | 20      | 83.1              |

\* A value less than the number of seedlings ...

From the observations it is clear that tomentolide B and marmelosin effectively inhibit the number of local lesions formed by SSMV. These coumarins are being further investigated for their inhibitory effect against a few more viruses both *in vitro* and *in vivo*.

Authors are thankful to Prof. T. S. Sadasivan for suggesting the problem.

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November 20, 1974.

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### OZONIUM WILT OF CROPS IN VARANASI

DURING the survey of diseases of field crops, severe incidence of a wilt and stem/root rot was noticed in several *kharif* and *rabi* crops on the Agricultural Farm from late September, 1973. Maturing plants showed wilting of the shoot with limp, drooping lower leaves; the soil moisture level was near saturation (80-85%) under moderately high temperature (28-30°C). Infection occurred near the soil level with the appearance of whitish ropy mycelial strands on the soil around, often overgrowing the stems. Consequently, the plants withered and died and the fungus made a profuse growth over the dead stems and branches, developing minute sclerotia on the fan-shaped mycelial strands.

The infection on the *rabi* crops usually occurred after the first irrigation or the winter rains, when the seedlings had entered the growth phase. Infected plants showed yellowing and withering of the foliage followed by a root rot. The roots were snapped at the mesocotyl and left in soil, when the plants were pulled out. The fungus showed scant growth and sometimes a few sclerotia on the wilted plants, but grew profusely when incubated under moisture at 30°C. A mortality of 10-12% in stand was observed in most of the crop hosts. Pre-emergence seed rot was also noticed in late plantings.

The tiny sclerotia from the infected hosts were separately surface-sterilized and washed in sterile water. A single sclerotium from each lot was planted in potato dextrose agar (pH 6.5) and incubated at  $30 \pm 1^\circ \text{C}$ . A 3-day plated colony showed typical fan-shaped, coarse mycelial growth aggregating into white, irregular hyphal knots over the branched, ropy strands (Fig. 1). A week old cultures showed profuse development of tiny, globulate, mustard brown sclerotia borne over the rhizomorphs in progressive developmental stages (Fig. 2). Cross and reciprocal inoculations of the host plants, raised separately under isolation, with the isolates indicated only one pathogen involved in the disease. Morphology, development and cultural characters of the pathogen indicated its identity to *Ozonium texanum* Neal and Wester var. *parasiticum* Thirumalachar<sup>3</sup>, to which it is referred.

The crops involved in the disease included green and black grams (*Phaseolus aureus* Roxb. and

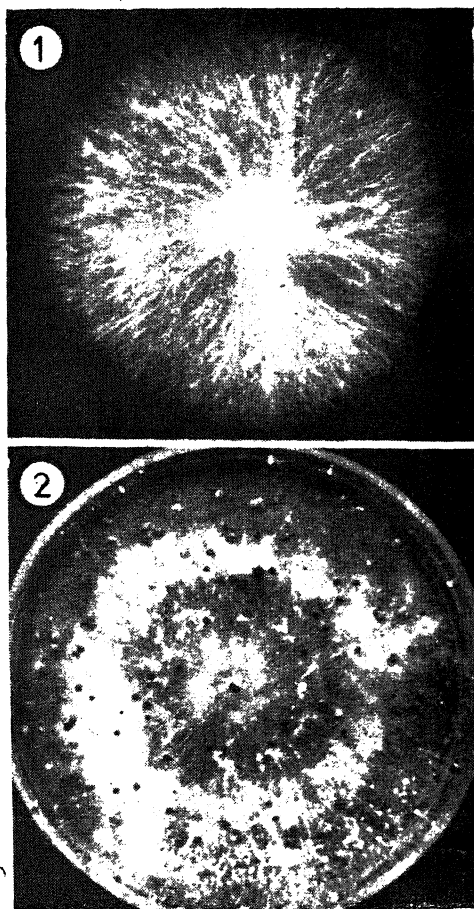
*P. mungo* L.), cowpea [*Vigna sinensis* (L.) Endl.] in the *kharij* season and gram (*Cicer arietinum* L.), oats (*Avena sterilis* L.), barley (*Hordeum vulgare* L.), flax (*Linum usitatissimum* L.) and potato (*Solanum tuberosum* L.) in the *rabi* season, of which gram and potato have been reported earlier as hosts from Bihar<sup>1-3</sup>. Parasitism of the fungus has not hitherto been reported on the other hosts. The species of the pathogen was originally reported from Bihar and Sub-Himalayan hills<sup>1-3</sup> and its occurrence here forms a new record for U.P. covering a wider host range.

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#### SPORIDIAL DISCHARGE IN *MELANOTAENIUM BRACHIARIAE*

THE follicolous smut *Melanotaenium brachariae* Viégas is fairly widespread in the country causing striate to blotchy, nonerumpent sori in the leaves and occasionally in the stems and rachis of *Brachiaria distachya* (L.) Stapf<sup>2</sup>. Morphology and cytology of teliospore germination and development of teliospores *in vivo* have been described earlier<sup>4</sup>. Teliospores from mature sori in the withering leaves stored at low temperature ( $12^\circ \text{C}$ ) were teased out and induced to germinate on slide mounts<sup>5-6</sup>. Mature sporidia floating on the water films were aseptically transferred to potato dextrose agar with 0.5% yeast extract (pH 6.5) and incubated at  $28^\circ\text{--}30^\circ \text{C}$ . Presoaked teliospores also were induced to germinate on plated PDA and those bearing young sporidia were transferred to PDA and incubated likewise. Association of compatible sporidial complements was ensured in both the culture isolates. The sporidia soon fused in compatible pairs and dikaryotic infective hyphae developed into small colonies. The colonies were slow in growth (1.5–2 cm diam./week), wrinkled, thick, dull chalky white and smooth, irregular in margin. They soon produced subcylindrical to allantoid secondary sporidia ( $3\text{--}8 \times 1.4\text{--}2.5 \mu$ ) in large numbers. The colonies were thus composed of dikaryotic hyphae and secondary sporidia.



FIGS. 1-2. Plated colonies (3-day and 1 week old) of *Ozonium texanum* var. *parasiticum*,  $\times 1$ .



Inoculation of cultures for 24-36 hr showed a number of young colonies scattered around the parent colony. Subcultures also showed several small young colonies on PDA, both on agar and on rice plate. These obviously appeared from the secondary sporidia (buds) later on, shot out from the young colony. The secondary colonies were circular, pale buff with striate margin, slightly raised at the centre and composed of young budding sporidia; they soon enlarged and developed similarly as the parent colonies (Fig. 1).



FIG. 1. Young colonies by expelled sporidia of *Melanotaenium brachiariae* on PDA.

Mature smut sor on the host parts go down to the soil with the withering leaves and gradually become released from the decomposing debris. The agglutinated teliospores in the sori get separated with the onset of monsoon rains, germinate and build up dikaryotic mycelial sporidial colonies on the soil underneath the host plants. The dikaryotic secondary sporidia bring about fresh infections on the young leaves late in August every year. Young grey sori become visible on the maturing leaves in early September.

Forcible discharge of infective or disseminative propagules is known to occur in several members of Basidiomycetes such as *Sporobolomyces*ales, *Dacrymyces*ales, *Uredinales* and *Ustilaginales*<sup>2</sup>. Several species of *Tilletia* Tul. and *Enyptoma* de Bary in the family *Tilletiaceae* (*Ustilaginales*) have been observed to expel their sporidia, tenderly

supported over the sterigmatal apices<sup>2</sup>. Forcible expulsion of sporidia was hitherto not reported in the genus *Melanotaenium* de Bary as by *M. brachiariae*.

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#### IS GREEN ISLAND IN *SORGHUM* A LIGHT MEDIATED REACTION?

THE "green island" reported in *Sorghum* is found in old rust infected leaves<sup>1</sup>. In the present studies, it is attempted to find out whether this reaction is due to differential penetration of sunlight through the canopy of *Sorghum* crop.

Four popular lines of *Sorghum*, viz., CSH-1, 370, 148 and CS 3541 were selected for the studies. Different densities of the plant population were maintained by varying the distance between rows, viz., 45 cm, 60 cm, 90 cm and 120 cm. The size of the plots was 7.2 m × 10 m. The experiment was replicated four times. Measurement of light (ft-c) was carried out at noon from 12 to 1-30, when there was maximum intensity of light over the canopy and minimum disturbance of the canopy due to wind. A Weston light meter (Weston Instruments, Inc., New York) model 756 with photo-selenium cells and quartz filter was used for the light measurements. The measurements were taken when the crop was in the grain filling stage, when there was the maximum intensity of rust infection.

The light intensity was measured on top of the canopy, bottom of the canopy and middle of the canopy, at three different places in a plot. Per cent penetration was derived based on the reading on top of the canopy. The per cent penetration at the bottom and middle of the canopy was averaged to get the overall light penetration within the canopy. To find out the correlation between light penetration and green island reaction, the number of leaves showing green islands from the base of the plants was counted.

TABLE I

Relationship of light penetration through Sorghum canopy to green island formation

| Sorghum line | Distance between rows in cm |   |     |   |     |   |     |   |
|--------------|-----------------------------|---|-----|---|-----|---|-----|---|
|              | 45                          |   | 60  |   | 90  |   | 120 |   |
|              | A                           | B | A   | B | A   | B | A   | B |
| CSH-1        | 3.9                         | 2 | 3.7 | 2 | 6.7 | 2 | 6.4 | 2 |
| 370          | 2.8                         | 3 | 3.8 | 3 | 3.7 | 3 | 5.7 | 3 |
| 148          | 3.1                         | 5 | 5.0 | 4 | 5.5 | 5 | 6.6 | 5 |
| CS 3541      | 5.5                         | 4 | 5.6 | 4 | 6.0 | 4 | 7.1 | 4 |

A=Per cent penetration of light.

B=Number of leaves showing green islands.

The data presented in Table I show that line 370 allowed the least amount of light to pass through the canopy, yet it had only 3 leaves from the bottom showing green islands. The line CS 3541 allowing greater amount of light to pass through had more number of leaves showing green islands. Within the variety, the different spacing of rows which allowed differential penetration of light did not influence the number of leaves manifesting green islands. Thus it becomes obvious that green island formation in *Sorghum* is not a light mediated reaction. In green island formation the activity of the rust gets confined to the ring of chloroplast surrounding the pustule. The chlorosis of the rest of the leaves probably limits the further spread of the fungus. The hybrid CSH-1 and 370 are susceptible to rust and yet only 2 to 3 leaves from the base exhibited green island formation. The varieties 148 and CS 3541 are highly tolerant to rust infection and 4 to 5 leaves from the base manifested green island formation in them. Thus it becomes quite apparent that green island formation is inversely related to the reaction of *Sorghum* to rust. Green island reaction is used as an index to assess host parasite compatibility in wheat rust<sup>2</sup>. This could very well be used in estimating the reaction of *Sorghum* lines to *Puccinia purpurea*.

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## EFFECT OF INDIUM ON CRYSTALLISATION OF SELENIUM

SELENIUM is a semiconductor and has several important applications. The growth of hexagonal selenium from melt is, however, hampered by a more easily growing amorphous phase. Single crystal growth takes place at extremely slow growth rates of  $10^{-7}$  cm/sec<sup>1</sup> or at pressures of five kbar<sup>2</sup>. Both these conditions are inconvenient. A breakthrough in selenium growth was achieved by Keezer<sup>3</sup> who found that addition of impurities makes it possible to grow single crystals of selenium under moderate vacuum and at reasonable growth rates. Keezer tried small concentrations of Na, K, Cl<sub>2</sub>, Br<sub>2</sub>, I<sub>2</sub> and Tl. Out of these, K, Cl<sub>2</sub>, Br<sub>2</sub> and Tl induced single crystal growth. These impurities reduce the viscosity of the melt and impede the formation of the Se<sub>n</sub> rings which constitute the amorphous phase. The absence of any effect in the case of I<sub>2</sub> was attributed by Keezer to the near-zero electronegativity difference between the I and Se atoms.

Indium belongs to group IIIA and the electronegativity difference between In and Se is slightly greater than that between Tl and Se. As such, indium should help the crystallisation of selenium. We have examined the effects of addition of indium on the crystallinity of selenium and some of the results are reported here. Filings of pure indium were added to pure selenium powder. Samples with different indium concentrations were taken in sealed pyrex tubes. The samples were heated to a temperature above the melting point of selenium and the melt was shaken thoroughly for some time with the help of an electromagnetic vibrator. The melt was then cooled. The sample in the pyrex tube was then kept in a Bridgman growth apparatus similar to the one described earlier<sup>4</sup>. Growth was carried out at the rate of 1.2 cm/hr.

The ingots were then fractured and the resulting surfaces were examined under a metallurgical microscope. The pure Se ingots showed irregular fracture. As the In content increased the ingots showed greater tendency towards cleavage. Also, while the fractured surface of pure Se ingot had a dull appearance, the surfaces of In-doped ingots showed increasing reflectivity. Detailed examination of the surface revealed that the entire ingot was not a single crystal but contained several small oriented single crystals. A typical crystallite with a stepped surface is shown in Fig. 1. The region in between these crystallites is non-reflecting and represents, presumably, amorphous Se. The crystallinity (crystallites-to-amorphous-phase ratio) increased with In concentration upto 1% In. At higher In

concentration, spherical globules were observed on the crystalline surface, indicating segregation of excess In. X-ray diffraction showed that the crystalline material is hexagonal Se. The 'cleaved' surfaces were etched with an etchant of composition  $(1/3) \text{H}_2\text{SO}_4 + (1/3) \text{HNO}_3 + (1/3) \text{H}_2\text{O}$  and showed etching characteristics similar to those observed by Harrison and Tiller<sup>2</sup> on the  $(10\bar{1}0)$  cleavage plane.

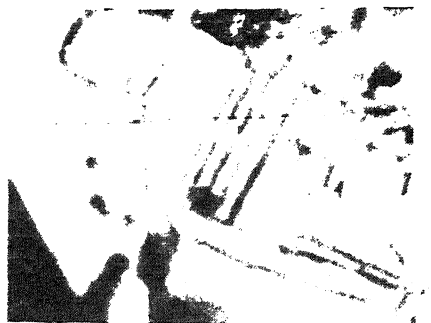


FIG. 1. Photomicrograph showing a crystallite of hexagonal Se grown from In-doped Se melt.

In conclusion, it has been found that the presence of small amounts of indium in selenium melt, induces single crystal growth of hexagonal selenium. The crystallinity improves with In concentration upto 1%. It is hoped that growth at smaller growth rates will result in larger crystal size. Work along these lines is on hand.

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## OCCURRENCE OF MULTINUCLEATE FUSIFORM INITIALS IN *SOLANUM MELONGENA* L.

WHILE studying the development of the vascular tissue differentiation in the stem of two species of the family Solanaceae—*Capsicum annuum* L. (chilli) and *Solanum melongena* L. (brinjal)<sup>1</sup>, an interesting feature was noticed in the fusiform initials in brinjal. Many fusiform initials showed the multinucleate condition (Fig. 1 A, at arrows). Generally two to five, or rarely more, nuclei per fusiform initial were found. Each nucleus had a distinct nuclear membrane and one to three nucleoli. The origin of the multinucleate condition has not been worked out so far. During the differentiation of the fusiform initials into vascular elements degeneration of one but all nuclei occurs. In the process of degeneration of the nucleus, the nuclear contents get degenerated first followed by the degeneration of the nuclear membrane. Or, sometimes, the spherical nucleus first becomes spindle-shaped due to elongation. The ends of the spindle-shaped nucleus become thread-like (Fig. 1 C)

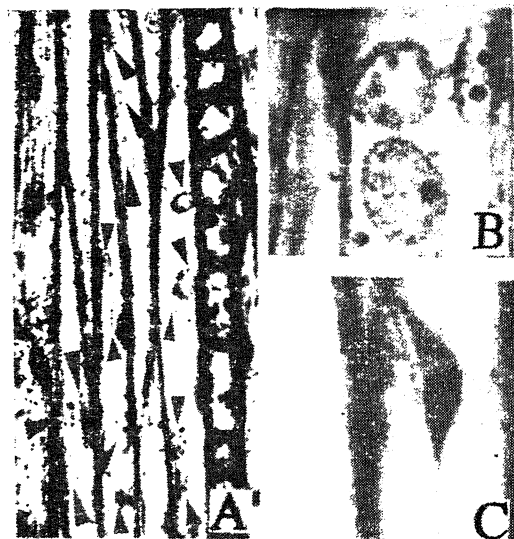


FIG. 1. A. Longisection stem: Note multinucleate condition of fusiform cells (at arrows),  $\times 175$ . B. Three nuclei seen in one focus,  $\times 975$ . C. Degenerating nucleus,  $\times 975$ .

and ultimately the tail of the nucleus gets degenerated. Gradually, the entire nucleus degenerates. Russow reported multinucleate cambial cells in pine (cf. Esau<sup>2</sup>). The possibility that the nuclei of a single fusiform initial belong to different cells lying one above the other (due to the thickness of the section) is ruled out for reasons that (a) most of

the nuclei are visible in a single focus or with a slight change in focus; (b) often the nuclei are found in a cluster, and all are in the same focus even under high magnification (Fig. 1 B); (c) the number of nuclei in the differentiating fusiform cells in the same section (of the same thickness) gradually decreases, and finally only one nucleus in a cell remains. Further ontogenic studies on this line may throw light on the origin of the multinucleate condition of fusiform initials.

I am grateful to Professor J. J. Shah for his interest in the work. This work was carried out during the tenure of a Jr. U.G.C. Fellowship.

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#### GENETIC TRANSFORMATION OF CLOVER INFECTIVITY MARKER (*clo*<sup>+</sup>) FROM *RHIZOBIUM TRIFOLII* TO *R. JAPONICUM*

THERE are several reports in the literature of DNA transfer<sup>1-6</sup> and episomal transfer<sup>7</sup> in *Rhizobium* for various genetic markers. Transformation has also been used to transfer plant tumour-inducing ability into *Rhizobium* from the crown-gall bacterium, *Agrobacterium tumefaciens*<sup>8-9</sup>. The present report describes an interspecific transfer of clover infectivity (*clo*<sup>+</sup>) from *Rhizobium trifolii* to *R. japonicum*, a species which does not nodulate the Egyptian clover (*Trifolium alexandrinum*).

The donor strain *R. trifolii* RT/3 (*clo*<sup>+</sup>) was an ultraviolet induced streptomycin resistant (30 µg/ml) mutant (*Str-r*)<sup>10</sup>, specific for *Trifolium alexandrinum* variety tetraploid C. The recipient *R. japonicum* 110 (*soy*<sup>+</sup>) supplied by Mr. V. Balasundaram was sensitive to streptomycin (*Str-s*) and specific for *Glycine max* variety Bragg. The transforming DNA from the donor was isolated according to the method of Marmur<sup>11</sup>. Since a direct identification of *clo*<sup>+</sup> hybrids on plates is difficult, streptomycin resistance marker from the donor was used to select the *R. japonicum Str-r* hybrids which were subsequently screened by plant nodulation test for inheritance of *clo*<sup>+</sup>.

Transformation was carried out by adding 0.5 ml of the donor DNA to 0.5 ml of the competent recipient culture (10<sup>8</sup> cells) (final DNA concentration 20 µg/ml) to make the final volume to 1 ml

and incubating the mixture for 30 min at 30 ± 1° C. After stopping the reaction, the mixtures were plated on yeast extract mannitol medium containing 30 µg streptomycin/ml to select the drug resistant transformants and on plain yeast extract mannitol agar for total counts. The controls included plating the culture without DNA treatment, recipient treated with recipient DNA, plating of the donor DNA alone and addition of DNase (50 µg/ml) before adding DNA. The competence of the recipient population for transformation was found to be at a maximum at the late log phase of its growth and the time during which an individual bacterium remained competent was about 72 min. *Str-r* colonies appeared at a frequency of 0.58%. Phenotypically *Str-r* colonies were checked for their drug stability by periodic transfers in drug-free and drug containing medium. The *Str-r* transformants were tested for nodulation on *Trifolium alexandrinum* under controlled conditions in agar test-tubes and eight out of thirty *Str-r* transformants nodulated this host legume (Table I). The *R. japonicum clo*<sup>+</sup> transformant I (Table I) was reisolated from the nodules formed by it on *T. alexandrinum* and was

TABLE I

*Nodulation of Trifolium alexandrinum (tetraploid C) by Rhizobium trifolii RT/3, R. japonicum 110 and R. japonicum clo*<sup>+</sup> *transformants on seedling agar (values average of 25 plants; nodule numbers were scored at the end of the sixth week)*

| Strains                                | No. of nodules/plant |
|--|----------------------|
| <i>R. trifolii</i> RT/3 (donor)        | 3                    |
| <i>R. japonicum</i> 110 (recipient)    | 0                    |
| <i>clo</i> <sup>+</sup> transformant A | 2                    |
| <i>clo</i> <sup>+</sup> transformant B | 1                    |
| <i>clo</i> <sup>+</sup> transformant C | 3                    |
| <i>clo</i> <sup>+</sup> transformant F | 2                    |
| <i>clo</i> <sup>+</sup> transformant I | 2                    |
| <i>clo</i> <sup>+</sup> transformant K | 1                    |
| <i>clo</i> <sup>+</sup> transformant Q | 2                    |
| <i>clo</i> <sup>+</sup> transformant R | 1                    |

found to nodulate both *T. alexandrinum* and *Glycine max* on seedling agar as well as in pots containing sterilized soil (Table II). This indicates that this transformant harboured both the *clo*<sup>+</sup> and *soy*<sup>+</sup> infectivity markers.

TABLE II

*Nodulation of Trifolium alexandrinum (tetraploid C) (clover) and Glycine max (Bragg) (soybean) by R. japonicum clo<sup>+</sup> transformant strain I 4, reisolated from the nodules formed by it on T. alexandrinum (values average of 25 plants; nodule numbers scored at the end of the sixth week)*

| Strains                                 | No. of nodules/plant |         |         |         |
|---|----------------------|---------|---------|---------|
|   | on seedling agar     |         | in pots |         |
|   | clover               | soybean | clover  | soybean |
| <i>R. trifolii</i> RT/3                 | 3                    | 0       | 9       | 0       |
| <i>R. japonicum</i> 110                 | 0                    | 2       | 0       | 7       |
| <i>R. japonicum clo<sup>+</sup></i> I 4 | 4                    | 3       | 7       | 11      |

Apart from host specificity, the donor *R. trifolii* RT/3 differed from the recipient *R. japonicum* 110 only in its resistance to streptomycin and its inability to ferment xylose. All the *R. japonicum clo<sup>+</sup>* transformants were resistant to streptomycin and like the recipient strain, all could ferment xylose.

The transfer of infectivity markers between different cross inoculation groups of *Rhizobium* opens out possibilities of evolving hybrid strains which could nodulate a wide range of legumes.

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## PHYLOGENY OF FLORAL NECTARY IN CONVULVULACEAE

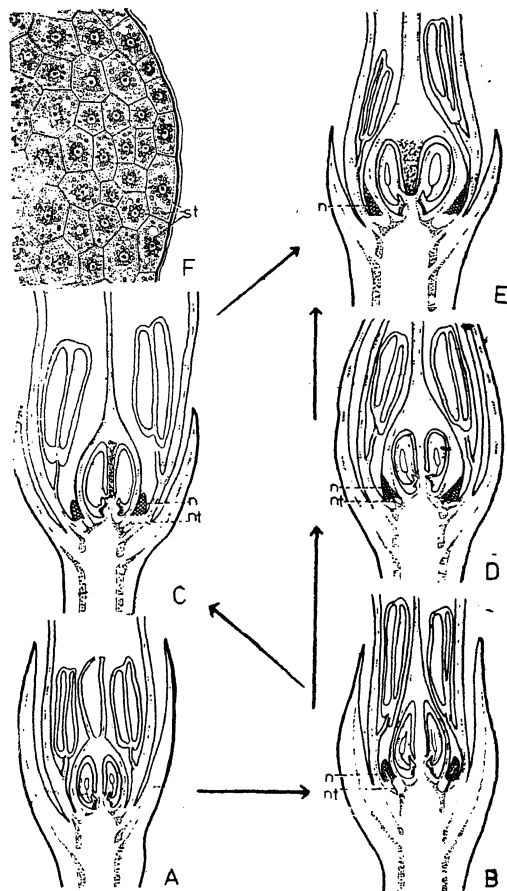
GOVIL (1972) while describing the floral anatomy of members of Convolvulaceae has interpreted the disc-like nectary as receptacular in nature. Morphology and anatomy of flower of Convolvulaceae have also been discussed by Tiagi (1966). Fahn (1953) has discussed at length the phylogeny and classification of floral nectaries in angiosperms. The present studies are confined to phylogeny of the nectary of Convolvulaceae.

Most of the members of the family are characterised by the presence of a discoid nectary surrounding the base of the ovary or forming a cushion. In genera like *Ipomoea*, *Argyrea*, *Rivea*, *Lettsomia* and *Convolvulus*, the discoid nectary is five lobed at the rim. It is fused at the base with the ovary and is free above. In *Convolvulus* the fusion of the disc with the ovary wall extends quite upward (Fig. D). Such a disc, however, is absent in genera like *Evolvulus*, *Cressa* and *Porana*. In *Merremia*, *Jacquemontia*, and *Cuscuta*, the disc cannot be differentiated from outside and remains fused with the ovary wall. The disc shows densely cytoplasmic cells with prominent nuclei. The cells have two or three small vacuoles and reserved food material in the form of mono- and disaccharides. In *Cuscuta*, these cells are packed with starch grains (Fig. F).

A study of the vascular supply of these species (Figs. A-E) shows that in members of *Ipomoea*, *Rivea*, *Argyrea*, and *Lettsomia*, the disc receives ten bundles which run up to its apex. In *Convolvulus*, the supply is feeble and extends up to half the height of the disc. In *Merremia* and *Jacquemontia*, the vascular supply is normal but the nectary is fused with the ovary wall. The disc in *Cuscuta* does not get any vascular supply.

On the basis of the morphological, anatomical and vascular supply of these nectaries in the members of Convolvulaceae, the phylogenetic relationships of nectaries have been derived. Members like *Evolvulus*, *Porana* and *Cressa*, where the nectary is absent are considered as primitive (Fig. A). Their primitiveness is also supported by the simple vasculature of their floral parts (Govil, 1972). Nectary in members of *Ipomoea*, *Argyrea*, *Rivea*, and *Lettsomia*, has evolved as an independent receptacular organ, receiving its vascular supply directly from the central vascular cylinder (Fig. B). During the course of evolution, nectary showed congenital fusion with the ovary wall and the vascular supply also started receding. The fusion of the disc and receding of the vascular supply, may take place simultaneously as in *Convolvulus* (Fig. C) or only the congenital fusion takes place without any change in the vascular

supply as in *Merremia* and *Jacquemontia* (Fig. D). Further evolution is marked by complete fusion of the nectary and absence of any vascular supply as in *Cuscuta* (Fig. E). Discussing the evolution and phylogeny of nectaries in angiosperms, Fahn (1953) has suggested "migration of the nectary from the perianth acrocentripetally to the ovary". Accordingly the nectary in *Cuscuta* may be considered as more advanced.



FIGS. A-F. Figs. A, B, C, D, E. Longitudinal sections of the flower buds respectively of *Evolvulus alsinoides*, *Ipomoea quamoclit*, *Convolvulus arvensis*, *Merremia emarginata* and *Cuscuta europaea* showing the position and vasculature of nectary (n—nectary; nt—nectary trace). Fig. F. A portion of the nectary of *Cuscuta europaea* showing starch grains (st—starch grains). Arrows indicate the lines of evolution.

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# CHANGES IN TOTAL AND SOLUBLE NITROGEN CONTENTS OF JUTE PLANTS FOLLOWING INFECTION BY *MACROPHOMINA PHASEOLI* (MAUBL.) ASHBY

CONSIDERABLE enhancement of total nitrogen content in host-pathogen complex in the early stages has been reported in a number of virus diseases as well as in potato infected with *Synchytrium*<sup>1</sup> and several other hosts infected with rusts and powdery mildews<sup>2</sup>. Such increase may be due to an increase in the protein synthesis by the pathogen<sup>3</sup>. Many fungal infections also stimulate greater protein synthesis in the host than can be accounted for, by the pathogen themselves. As regards changes in soluble nitrogen, as a result of disease development, conflicting views (both increase and decrease) are reported<sup>4,5</sup>. In the majority of the cases, however, at very late stages of disease, the total nitrogen content of the fungus infected plant organs, usually decreases<sup>6</sup>. This decrease is associated with the decomposition of proteins, mainly due to the breakdown of the cell structure of infected tissues and correlated with an increased activity of the proteolytic enzymes. The present investigation has been undertaken with a view to estimating quantitatively the total and soluble nitrogen contents of the stems (2nd to 5th internode) of healthy and *Macrophomina*-infected jute plants (*Corchorus capsularis* L.) of different ages.

In analysing total nitrogen the method described by Vogel<sup>7</sup> (1961) and modified by Gupta<sup>8</sup> (1970) was mainly followed. 15 mg of dry tissue was digested with 2 ml of analar sulphuric acid for 45 min in a micro-Kjeldahl flask. About 0.8 to 1 ml of hydrogen peroxide (30%) was added to decolorize the digested material and the final volume, after decolorisation, was made upto 100 ml with distilled water. To 1 ml of the above aliquot 1 ml of a mixture of 10% sodium silicate and 10% sodium hydroxide (1 : 1) and 5 ml of Nessler's reagent were added. After 10 to 15 min the intensity of the yellowish brown colour was measured at 430 mμ in a 'Hilger pattern biochemical absorptiometer'. A blank set was maintained with 1 ml of analar sulphuric acid treated in an identical manner. The total nitrogen content was expressed as mg/gm dry weight of tissue, by comparing the observed values

with standard curve made from analar ammonium sulphate.

For estimating soluble nitrogen, 500 mg of fresh tissue was homogenised with 5-8 ml of distilled water and it was kept overnight in ice chamber after the addition of 1 ml of 50% TCA. It was centrifuged and the volume of the supernatant was made upto 10 ml with distilled water. Then it was digested with analar sulphuric acid and similar procedure was followed as in the case of determination of total nitrogen.

TABLE I

Total and soluble nitrogen contents of healthy and Macrophomina-infected jute plants

| Age of the plants (in month) | Total nitrogen content in mg per gm of dry tissue |          |                      |  | Soluble nitrogen content in mg per gm of dry tissue |          |                      |  |
|------------------------------|---|----------|----------------------|--|---|----------|----------------------|--|
|                              | Healthy   | Infected | Gain (+) or Loss (-) |  | Healthy   | Infected | Gain (+) or Loss (-) |  |
| 1.0                          | 7.1   | 10.2     | +3.1                 |  | 2.4   | 2.2      | -0.2                 |  |
| 1.5                          | 8.4   | 11.8     | +3.4                 |  | 2.9   | 2.5      | -0.4                 |  |
| 2.0                          | 10.1  | 14.2     | +4.1                 |  | 3.2   | 2.6      | -0.6                 |  |
| 2.5                          | 13.1  | 18.2     | +5.1                 |  | 4.5   | 3.4      | -1.1                 |  |
| 3.0                          | 15.7  | 21.8     | +6.1                 |  | 5.2   | 3.8      | -1.4                 |  |
| 3.5                          | 15.9  | 17.4     | +1.5                 |  | 5.07  | 4.1      | -1.6                 |  |
| 4.0                          | 15.9  | 13.5     | -2.4                 |  | 4.7   | 3.8      | -0.9                 |  |

The results show that the total nitrogen content of the tissues of healthy jute plant increases gradually with age upto three months after which it more or less remains uniform. Infected tissues, however, show considerable increase in total nitrogen even in one month old plants. With further aging, the infected plants also show gradual increase in total nitrogen upto three months, and then it suddenly decreases. Soluble nitrogen content gradually increases in healthy plant tissues with age upto three months. With further aging the amount of soluble nitrogen decreases slightly. In the infected plants, however, the tissues show a decrease in soluble nitrogen content from those of healthy plants at all stages of development. The loss of soluble nitrogen gradually rises with age and reaches maximum in the three month old plants. The gradual decrease in soluble nitrogen content, simultaneously with increase in total nitrogen content may possibly be due to utilization of the former by the pathogen, while enhancement of the latter may be accounted for the stimulated protein synthesis of the host or pathogen or both.

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### GENETIC TRANSFER OF NITROGEN FIXATION GENE (*nif*<sup>+</sup>) FROM *AZOTOBACTER CHROOCOCCUM* TO *RHIZOBIUM TRIFOLII*

INTRASTRAIN genetic transfer of nitrogen fixation genes (*nif*<sup>+</sup>) has been accomplished by transduction<sup>1</sup> and conjugation<sup>2</sup>. This was followed by a report on intergeneric conjugational transfer of *nif*<sup>+</sup> from *Klebsiella pneumoniae* to *Escherichia coli*<sup>3</sup>. The present report deals with an intergeneric transfer of nitrogen fixation gene (*nif*<sup>+</sup>) by DNA transformation from the bacterium *Azotobacter chroococcum* to *Rhizobium trifolii*, a species which does not naturally fix nitrogen in the free living state.

The donor *Azotobacter chroococcum* B3 is a non-symbiotic nitrogen fixer, isolated from the rhizosphere of barley plants. The recipient *Rhizobium trifolii* RT/3 is an ultraviolet induced streptomycin resistant mutant (30 µg/1) of *R. trifolii* T5<sup>+</sup>. The transforming DNA was isolated from the donor according to the method of Marmur<sup>4</sup> and after isopropanol precipitation was stored in saline citrate over a layer of chloroform at 0° C.

Transformation was carried out by adding 0.5 ml of the donor DNA to 0.5 ml of the competent recipient cells (10<sup>6</sup> cells) to make the final volume to 1 ml (final DNA concentration 20 µg) and incubating the mixture for 30 min at 30 ± 1° C. After stopping the reaction, the mixture was plated on nitrogen free mannitol agar (20 g mannitol, 5 g CaCO<sub>3</sub>, 0.1 g K<sub>2</sub>SO<sub>4</sub>, 0.2 g K<sub>2</sub>HPO<sub>4</sub>, 0.2 g MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.2 g NaCl, 15 g agar, 1000 ml distilled water; pH 7.3) and the colonies were replicated on a series of silica plates fortified with the same nitrogen free medium. Controls included plating the recipient culture on silica plates without DNA treatment, recipient treated with the recipient DNA, plating the donor DNA alone, addition of DNase (50 µg/ml) before adding DNA and also on yeast extract mannitol agar for total counts.

The competence of the recipient population for transformation was found to be at a maximum at the late log phase of its growth and the time during which an individual bacterium remained competent was about 42 min. *R. trifolii* *nif*<sup>+</sup> transformants appeared at an average frequency of 0.37%. Phenotypically *nif*<sup>+</sup> colonies were further purified by repeatedly culturing them on nitrogen free silica plates and finally growing them in nitrogen free broth. These were tested for acetylene reduction in nitrogen free medium. Table I shows the specific acetylene reducing capacity of the *nif*<sup>+</sup> transformants, which have all been found to be active.

TABLE I  
Acetylene reducing activity of *A. chroococcum* B 3 and *R. trifolii* *nif*<sup>+</sup> transformants [Gas phase : argon : O<sub>2</sub> : acetylene 80 : 10 : 10 (v/v); incubation time 30 min ; 72 hr old cultures]

| Organisms                               | n mol ethylene formed/<br>30 min/mg<br>nitrogen |
|---|---|
| <i>A. chroococcum</i> B3 (donor)        | 50  |
| <i>R. trifolii</i> RT/3 (recipient)     | < 0.2   |
| <i>nif</i> <sup>+</sup> transformant A4 | 32  |
| „ B1                                    | 43  |
| „ B5                                    | 50  |
| „ B2                                    | 41  |
| „ A10                                   | 38  |

Like the recipient *R. trifolii* RT/3, the transformants nodulated *Trifolium alexandrinum* (variety tetraploid C). This property identifies them as strains of *R. trifolii* (Table II). The transformants also resembled the recipient in their serological properties, the details of which will be reported separately.

TABLE II  
Nodulation of *Trifolium alexandrinum* (variety tetraploid C) by *A. chroococcum* B 3 and *R. trifolii* *nif*<sup>+</sup> transformants (nodulation test done in test-tubes containing seedling agar and nodule number scored at the end of the 4th week ; values average of 8 replicates)

| Organisms                               | No. nodules/plant |
|---|-------------------|
| <i>A. chroococcum</i> B 3 (donor)       | 0                 |
| <i>R. trifolii</i> RT/3 (recipient)     | 6                 |
| <i>nif</i> <sup>+</sup> transformant A4 | 5                 |
| „ B1                                    | 3                 |
| „ B5                                    | 2                 |
| „ B2                                    | 2                 |
| „ A10                                   | 4                 |

The symbiotic *Rhizobium* does not fix nitrogen in its free living state and the essential genes for

nitrogen fixation are considered to be activated by compounds present in the microenvironment of the plant nodules. The establishment of *nif*<sup>-</sup> genes in this bacterium, therefore, opens out many questions on symbiosis for further investigation, besides conferring an ecological advantage for their survival in natural environments.

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#### CHANGES IN NUCLEIC ACID LEVEL IN JUTE STEMS DUE TO INFECTION BY *MACROPHOMINA PHASEOLI* (MAUBL.) ASHBY

THE subject of nucleic acid metabolism in obligate parasitism has been elegantly reviewed<sup>1</sup>. Parasites, with compatible combinations in the susceptible hosts, show a marked increase in the amount and the activity of RNA in infected tissues<sup>2-4</sup>, while with resistant hosts, they do not show such increase. The increase in RNA content, in response to host parasite interaction, is possible due either to increased synthesis of certain RNA molecules or new RNA species or both. The DNA content, on the other hand, does not show any appreciable changes in many cases. It has been found that in compatible wheat infected with *Puccinia graminis*, the amount of DNA remains almost the same or slightly less in diseased than in the healthy tissue<sup>5-6</sup>. An enhancement of DNA, RNA and protein content at the early stages of club root of cabbage hypocotyls by *Plasmodiophora brassicae* has been reported recently<sup>7</sup>.

The present investigation has been undertaken to study the quantitative changes in DNA and RNA contents of the stems (2nd to 5th internode) of healthy and *Macrophomina phaseoli* (Maubl.) Ashby infected jute plants of different ages. Jute plant (*Corchorus capsularis* L.) is attacked by *Macrophomina phaseoli* causing great loss in both quantity and quality of the fibre.

The total nucleic acid content was extracted following mainly the method described by Cherry<sup>8</sup>. In analysing DNA level, the diphenylamine method



the amount of DNA in the infected tissues of wheat plants. Markham (1966) has reported a decrease in the amount of DNA in the infected tissues of wheat plants.

The amount of RNA in the infected tissues of wheat plants also decreases with the development of the disease and the infected tissues become more degenerated.

TABLE I

Changes in the amount of DNA and RNA in the infected tissues of wheat plants during the development of the disease

| Age of the plants in days | DNA content (mg/gm dry weight) |          |         |                       | RNA content (mg/gm dry weight) |          |         |                       |
|---------------------------|--------------------------------|----------|---------|-----------------------|--------------------------------|----------|---------|-----------------------|
|                           | Healthy                        | Infected | Control | Loss due to infection | Healthy                        | Infected | Control | Loss due to infection |
| 15                        | 1.41                           | 1.37     | 1.45    | 0.08                  | 1.29                           | 1.21     | 1.35    | 0.14                  |
| 30                        | 1.42                           | 1.41     | 1.48    | 0.07                  | 1.30                           | 1.25     | 1.40    | 0.15                  |
| 45                        | 1.41                           | 1.40     | 1.48    | 0.08                  | 1.29                           | 1.24     | 1.38    | 0.14                  |
| 60                        | 1.44                           | 1.43     | 1.47    | 0.03                  | 1.32                           | 1.27     | 1.42    | 0.10                  |
| 75                        | 1.43                           | 1.42     | 1.47    | 0.05                  | 1.31                           | 1.26     | 1.41    | 0.10                  |
| 90                        | 1.44                           | 1.43     | 1.47    | 0.04                  | 1.32                           | 1.27     | 1.42    | 0.10                  |
| 105                       | 1.44                           | 1.43     | 1.47    | 0.04                  | 1.32                           | 1.27     | 1.42    | 0.10                  |
| 120                       | 1.44                           | 1.43     | 1.47    | 0.04                  | 1.32                           | 1.27     | 1.42    | 0.10                  |

plants vary slightly. In mature tissues the amount increases slightly with an increase in age of the plants upto 2 months. With further increase in age, the amount of DNA decreases slightly. The loss of DNA content increases slightly with the disease development and at the end of the experimental period the loss of DNA content becomes well defined in the tissues. This is in accordance with the earlier observations in wheat varieties having compatible reaction with *Puccinia graminis* which shows that DNA contents remain slightly less or almost unchanged.

In contrast to the changes in DNA the RNA increases in the tissues of the infected plants in the early stages of the disease. But a decline in the RNA contents in the infected tissues is prominent in the three month old plants. The decrease in RNA becomes more prominent with further aging of the plant. The initial increase in RNA is in accordance with similar observations made earlier in a number of cases (1967). The decrease in both DNA and RNA at some advanced stages of the disease may be due to denaturation of nucleic acids associated with degeneration of cellular components of the host tissues.

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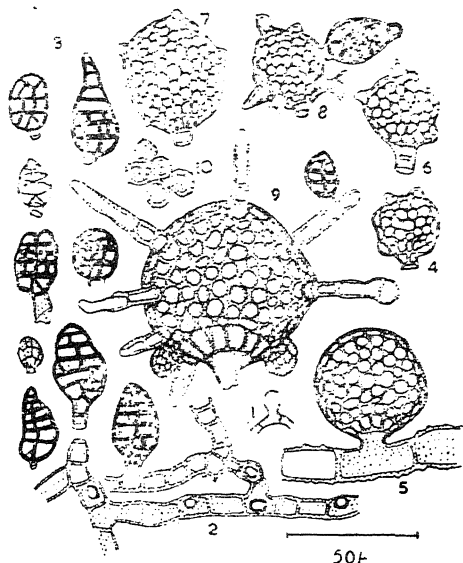
## A NEW SPECIES OF *PITHOMYCES* WITH BULBILS

In the course of his studies on Hyphomycetes of Bhawal the author collected an interesting species of *Pithomyces* Berk and Br., on dead bark of *Eucalyptus* species. The morphological characters of the fungus are given below.

Colonies: dark blackish brown to black, irregular and often effused. Mycelium partly immersed but mostly superficial, composed of a network of branched and anastomosing, subhyaline or brown, smooth or occasionally verruculose; conidiophores borne laterally on the hyphae, simple, straight, occasionally curved, continuous, subhyaline to light brown, concolorous with hyphae, 3-10.5  $\mu$ m long, 3-5  $\mu$ m thick. Conidia formed singly as blown out ends at the apex of each conidiophore, straight or curved, usually obelovate or obpyriform, sometimes sub-spherical or oval, muriform, 5-18 celled, with the cells arranged in 3-8 transverse rows, smooth-walled, brown or dark brown, 20-38  $\mu$ m long, 15-23  $\mu$ m thick in the broadest part. Bulbiliophores borne laterally on the hyphae, simple, straight, cylindrical, hyaline or subhyaline, 7-13.5  $\mu$ m long and 6.5-15  $\mu$ m thick; bulbils generally globose, 27-50-75  $\mu$ m in diam, brown to dark brown, smooth or tuberculate or with 5-20 radial appendages, sometimes with multicellular tuberculate outgrowths at basal part around bulbiliophores; appendages stiff, straight, hypha-like, 0-5 septate, 20-55  $\mu$ m long, 5-7  $\mu$ m wide, simple, rarely once branched at the apex, sometimes bearing conidia terminally or laterally. The conidia and bulbils usually become detached through fracture of the wall of the conidiophore and each conidium carries away with it the upper part of the conidiophore.

The present species of *Pithomyces* Berk and Br. differs from all other taxa classified in this genus from the characteristic stipitate bulbils and conidia.

The specimen was examined by Dr. M. B. Ellis who considers it to be a new species of *Pithomyces*. It is, therefore, being described here as a new species.



FIGS. 1-10. *Pithomyces bulbilius*. Fig. 1. Developing conidium. Fig. 2. Hyphae with a conidium in side view and five scars of broken conidiophores in top view. Fig. 3. Conidia. Figs. 4-9. Stipitate bulbili. Fig. 10. Cells in a small fragment of bulbil.

*Pithomyces bulbilius* Satya sp. nov.

Coloniae effusae, fuscae vel atrae, irregulares. Mycelium superficiale ex hyphis septatis, subhyalinis vel brunneis, levibus, rarius verrucosis, 3-7-5-15 µm crassis, reticulatis compositum: conidiophora singula ex lateribus hypharum oriunda, simplicia, recta, cylindrica, continua, subhyalina vel pallide brunnea, 3-10-5 µm longa, 3-5 µm crassa: conidia singula in apice conidiophori oriunda, recta vel curvata, obclavata vel obpyriforma vel rotunda vel ovalia, dictyospora, 5-18 cellularia, vulgo cellulis in 3-8 ordines transversa depositis, brunnea vel atrobrunnea, levia, 20-38 µm longa, 15-23 µm crassa. Bulbilio-phora singula ex lateribus hypharum oriunda, simplicia, recta, cylindrica, hyalina vel subhyalina, 7-13-5 µm longa, 6-5-15 µm crassa, bulbilia vulgo globosa, 27-50-75 µm in diam., brunnea vel atrobrunnea, levia vel tuberculata vel cum 5-20 apendicibus; appendices rigidi, recti, similis hyphis, 0-5 septati, 20-55 µm longi, 3-5 µm lati, simplices, non-nunquam producentes conidia acropleurogena.

In cortice mortuis *Eucalypti* sp. leg. H.N.S. die 1 Januari, anni 1965, typus positus in C.M.I., Kew, sub numero IMI 111863.

The type specimen has been deposited in C.M.I., Kew, London, as No. IMI 111863. The author expresses his grateful thanks to Dr. S. B. Saxena, Department of Botany, University of Nagpur, for encouragement and to Prof. O. N. Handoo for facilities. He thanks Dr. M. B. Ellis, Director, C.M.I., Kew, for helpful suggestions and Rev. Fr. Devanand for Latin diagnosis.

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A NEW SPECIES OF *MASSARINA* SACC.

AUTHORS collected a species of *Massarina* on dried stem pieces of *Lantana camara* L., which differed from all the existing species in having 3-celled ascospores and uniseriate arrangement of ascospores. Hence it is being described here as a new species.

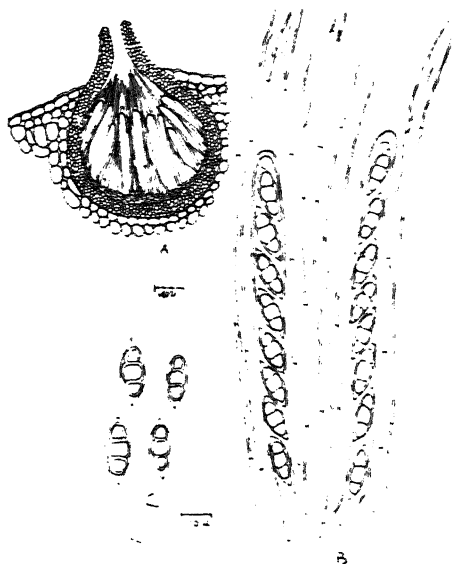


FIG. 1. A. Median L.S. Perithecium. B. Asci containing ascospores, paraphysoids. C. Ascospores with mucous sheaths.

*Massarina triculula* Sp. Nov. Panwar and Kaur

Ascocarpeae pyriformes, innatae, dispersae inter emortuorum ramunculorum corticem, rostrum nigrum, erumpens, 220-405 × 260-385 µ magnitudinis. Ascocarpeae quarum crassitudo parietis est amplitudinis 3-4 cellulae, habent crassos parietes, cellulae polyhedrae, et cellulae interiorae cavitatem versus

hyalinae et parvis granis. Aseel 8-sporati, breviter stipitati, sphaerici, obtusiusculi, apud apicem crassius, 1.5-1.55  $\mu$  diam. Vagina mucosa ascosporas tegens, ad apicem extremas acuta, oblique uniseriata, valvulata, hyalinae, 2-septatae, apud septa constrictae, 15-5.15  $\mu$  5.4-6.7  $\mu$ . Paraphysoidae numerosae, tenues, hyalinae, filiformes, septatae, parvis sepe tenues et numerosae, 1.4-4.0  $\mu$  in diam. Fig. 1 A, B and C.

Collected in India as *emertalis* *Lantana camara* L. Mount Abu, Rajasthan, VIII, 1974.

Specimen apud C.M.H. Kew, Herb. depositum IMI 18826 type, apud Botany Department, University of Jodhpur, Jodhpur, I.M.I.L. 359.

We are grateful to Dr. Shuen-an for the help in the identification of the fungus and to Prof. H. C. Arya for providing laboratory facilities. Thanks are also due to the Rev. Father William Burrage for the Latin diagnosis.

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## ENDOGENOUS GROWTH SUBSTANCE LEVEL IN SWEET ORANGE LEAVES AS INFLUENCED BY 'SANKUTEGULU'

A DISEASE locally known as 'Sankutevalu' of unknown etiology has been reported by Reddy and Murthi<sup>1</sup>. This disease is widely prevalent in Cuddapah and Anantapur Districts of Andhra Pradesh, causing heavy losses to the citrus crop. The chief symptoms are the yellowing and reduction in size of the leaves, premature leaf drop, distorted fruits and even small fruits turning dull yellow becoming stony and ultimately leading to the death of the tree. In the present investigation an attempt has been made to elucidate the effect of the disease on the endogenous growth substance levels in the leaves to understand as to what extent the promoter-inhibitor balance has been altered.

The material for the study was collected in a citrus orchard near Kadiri (A.P.) showing typical disease symptoms. Leaves from the healthy and diseased plants of the same age were collected and the endogenous growth substance levels were

estimated. The leaves were extracted as per the method of Goldschmidt and Monselise<sup>2</sup> and the rice coleoptile bioassay was carried out to assay the growth substance activity according to the method of Das et al. (1965). The results presented in the histograms are the mean of four independent estimations. The data were statistically analysed.

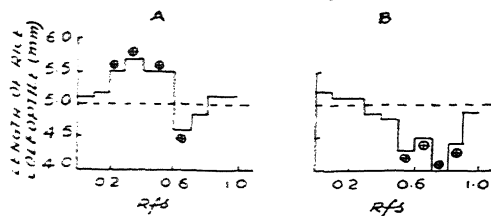


FIG 1

FIG. 1. Endogenous growth substance levels in (A) Healthy and (B) Diseased Sweet Orange leaves. Note: Dotted lines indicate the length of rice second leaf sheath in distilled water control.

B indicates significance at 1% level.

In the healthy leaves (Fig. 1 A), a significant promoting activity was noticed at  $R_f$  0.2-0.6, while it was not significant at  $R_f$  0.0-0.2. The inhibitory activity was significant at  $R_f$  0.6-0.7. In the diseased leaves (Fig. 1 B) significant promoting activity was not evident at any of the  $R_f$ . However, a band of significant inhibitory activity was seen at  $R_f$  0.5-0.9, partly corresponding to the  $\beta$ -inhibitor zone of 0.4-0.7. Thus a tilt in the hormonal balance towards the accumulation of inhibitors is apparently clear. The conspicuous absence of significant promoting activity in the diseased leaves as in the present study was also reported in mosaic injected Sathgudi leaves (Rao and Narasimham<sup>4</sup>). Hanks and Feldman<sup>5</sup> also observed a reduction in the endogenous promoter level in exocortis infected citrus terminals.

The first author is grateful to the C.S.I.R. for financial assistance.

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March 25, 1975.

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## SHORT SCIENTIFIC NOTES

Heterostyly in *Solanum khasianum* Clarke

*Solanum khasianum* Clarke is a wild plant which forms a rich source of the glyco-alkaloid solasodine used in the preparation of cortisone and other sex hormones. In a breeding programme aimed at crop improvement, the occurrence of flowers in which the styles were of different lengths on the same plant was observed to be a common feature of this crop. Studies of the frequency of occurrence of these flowers in relation to variety, age of the plant and fruit set are presented in this report.

Two varieties of *Solanum khasianum* were used in this study. One is a wild collection with straight spines and the other is a mutant with curved spines, obtained from the Bhabha Atomic Research Centre, Trombay.

The length of the style varied from less than a millimetre to as much as 15 mm. The variation was rather continuous but 2 broad categories could be recognised. In some flowers (long styled), the style is long and is clearly seen above the column of the anthers. In the second type (short styled), the style lies well below the column of the anthers.

The frequency of occurrence of these 2 types of flowers has been observed to vary with the age of the plant. When flowering started, the 2 types of flowers occurred approximately in equal frequencies. As the plant aged, there is a distinct increase in the frequency of the long styled flowers subject to minor fluctuations.

The functional normality of these 2 types of flowers was tested by making all possible crosses involving flowers of the same as well as different plants. The most successful crosses were those where the female had a long style. Fruit set was nearly 90% in such crosses, irrespective of the nature of the flower from which the pollen was used. There was practically no fruit set in crosses on short styles.

Heterostyly is a common feature observed in solanaceous species<sup>1-6</sup>. The observations on *Solanum khasianum* presented here are in agreement with those in brinjal and tomato, where fruit set is maximum in flowers with long styles<sup>3,4</sup>. However, in brinjal<sup>6</sup>, hand pollination of short styled flowers increases fruit set unlike in *S. khasianum*.

The causes of these variations are not clearly understood although the effect of weather and season are suggested to be the main factors<sup>4</sup>. The production of 2 types of flowers could be of evolutionary significance. In nature, the short styled

flowers should set fruit only from self-pollination, while the long styled ones could do so from either self or cross pollination. The phenomenon of heterostyly might have been evolved as a genetic homeostatic mechanism for the release and maintenance of genetic variability.

The authors are grateful to Dr. G. S. Randhawa, Director, for his interest and encouragement.

Indian Institute of Horticultural Research,  
Bangalore, January 17, 1975.

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Studies on the Rhizosphere Microflora of Ginger  
*Zingiber officinale* (L.) Rosc

During the course of investigation of rhizosphere mycoflora of ginger [*Zingiber officinale* (L.) Rosc] the following fungi were isolated. These fungi have not previously been reported from India<sup>1</sup>. Their diagnostic characters are as follows:

*Chaetomidium subfimetii* Seth. in *Trans. Brit. Mycol. Soc.* 50 (1), 45-47, 1967.

Colonies on PDA greyish black; perithecia without ostiole, globose, black, covered with loose mass of hairs, 160-275  $\mu$  diam. attached to the substratum by rhizoides, appendages of two types, dark brown, thick-walled, smooth to distinctly roughened, 5-5  $\mu$  wide, wider at the base, septate, unbranched, long, ending in a rounded hyaline tip; and yellowish brown, septate, very minutely roughened, straight, 3-4.5  $\mu$  wide, ending in a hyaline tip; asci club-shaped, situated at the base of the ascocarp, 8-spored, ascospores irregularly bi-serrately arranged, paraphyses filiform present only in the early stages, ascospores light to olive brown, broadly lemon-shaped, 7.5-9  $\times$  5.5-6.5  $\mu$ , slightly umbonate at both ends.

The culture has been deposited in the herb. I.M.I. No. 172195.

*Fusarium arthrosporioides* Sherbakoff in *Mem. Cornell agric. Exp. Sta.* 6, p. 175, 1915.

Aerial mycelium white with tinge of rose or salmon colour, substratum leather brown or light red, 1-celled conidia 4-6  $\times$  3-8  $\mu$ , 3-5 septate, spindle-to lance-shaped slender conidia, 35  $\times$

specimens were found to be larger, more or less pedunculate, with rounded apices—or hooked-shaped, frequently with a 42–44 µm long peduncle.

The material has been deposited in the herbar. I.M.I. No. 172171.

The authors are thankful to the Director, Commonwealth Mycological Institute, Kew, England, and his staff for their generous help in the identification and comments on the specimens.

Department of Mycology, and N. D. SHARMA  
Plant Pathology, L. K. JOSH, I.

J.N. Agricultural University,  
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### Efficacy of Resorantel (Terenol) Against Paramphistomiasis in Goats and Calves

Paramphistomiasis is considered to be a serious helminthic problem of Indian ruminants (Varma, 1957). The natural outbreaks of the disease in Bihar are often encountered particularly in flood affected areas every year, after rains from October to March. Large number of drugs has been tried for its treatment. The efficacy of Resorantel has been assessed in this note.

In the month of January, 33 goats 3 months of age were purchased from Patna for some experimental work but within a week these animals started showing symptoms of diarrhoea and bottle jaw. Within a week, 15 animals died and post-mortem examination revealed numerous immature and mature paramphistomes in the rumen, abomasum and duodenum assignable to *Campylophoron* spp. The remaining 20 animals showed similar typical symptoms of Paramphistomiasis. Faecal samples of all these animals were examined and 12 of them were positive for paramphistome eggs. To these 20 animals, a single dose of Resorantel, 2, 6-dihydroxybenzoic acid-4-bromanilide (Terenol)<sup>1</sup> was given. One ml suspension prepared from 9 g of the Resorantel powder in 100 ml water was administered per kg body wt. per os in the morning before feed (about 65 mg/ml active substance). Treated animals were observed daily for 30 days and weekly weight was recorded. After a week faecal samples of all the animals were found completely negative for paramphistome eggs. Weekly weight showed a gain of 0.3 to 0.8 kg per animal. After the

treatment, there was marked improvement in the general conditions of all the experimental animals and these recovered completely.

The drug was also tried on calves. Ten clinically positive cases of Paramphistomiasis were selected from an outbreak of the disease from Muzafferpur (Bihar) and were treated with Resorantel in similar doses. The animals were observed for a month and except 2, all calves recovered completely.

These observations suggest that Resorantel (Terenol), so far a cestocidal drug is also effective against Paramphistomiasis in goats and calves and this finding confirms the work of Gaenssler (1974) on *Paramphistomum microbothrium* in South Africa.

Disease Investigation. B. K. SINHA,  
Control and Livestock J. S. AHLUWALIA,  
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Bihar, Patna 800 014, March 21, 1975.

<sup>1</sup> Product of M. s. Hoechst AG and Behringwerke, Germany, made available by the kind courtesy of Dr. B. N. Sahai, Bihar Veterinary College, Patna.

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### Two New Records of Clupeid Fishes, *Ilisha kampen* and *I. sirishai* from the Arabian Sea

*Ilisha kampen* was originally described<sup>1</sup> as *Pellona kampen* from Java and Borneo. Norman<sup>2</sup> recorded this species from Madras, based on a single specimen in the British Museum, but it has been identified as *Ilisha megaloptera* by Whitehead (1941). Recently *I. kampen* has been recorded for the first time from the Bay of Bengal by the present author<sup>3</sup>. The species is now being recorded for the first time from the Arabian Sea based on 140 specimens collected at Bombay in January 1975 and identified as described earlier<sup>3</sup>; body depth 26.3–29.5 in % of S.L., D 16–18, P 14–16, V 7, A 42–45; gillrakers 10–11 + 21–23, scutes 18–20 + 8–9 (total 26–28). The large number of specimens show that the species is abundant in the region.

*Ilisha sirishai* has been described as new from Visakhapatnam on the east coast<sup>4</sup>. The species can be identified by the characters described earlier<sup>4</sup>; swimbladder without post-coelomic extension; body depth 30.0–35.35.2 in % of S.L., D 17–18, P 15–17, V 7, A38–44; gillrakers 10–14 + 24–27, scutes 18–20 + 9–10 (total 27–29). This species is now being recorded from Cochin based on 5 specimens (June 1973), and from Bombay based on 3 specimens collected during January 1975.

I am thankful to C. S. Rao, Indian Institute of Technology, Powai, Bombay, and to the Research Officer, Tanaporewala Marine Biological Station, Bombay, for their help in the collection of specimens.  
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#### Insect-pollination in Ber (*Zizyphus mauritiana* Lamk.)

Ber fruit contains appreciable amounts of vitamins 'A', 'B' and 'C'. It has more protein, calcium and vitamin C as compared with apple. The caloric value per 100 gm of the fruit has been reported to be 55 and it has 75-150 mg of vitamin C per 100 gm of the fresh fruit<sup>2</sup>.

The flowers of the Ber are cross-pollinated. Because of its pollen being heavy and thick in

nature, insect-like the honeybees, etc., apple and the house fly (*Musca domestica*) have been reported to play an important role in its pollination<sup>1</sup>. The present author while conducting survey on the insect-pests of forest trees during August-November, 1974 observed the yellow wasp, *Polybia dorsalis* (Fabricius) visiting ber flowers. This pollinator was abundant throughout the flowering period of this stone fruit and showed its maximum activity between 12-2 P.M. daily. It visited 18 flowers per minute. It is thus a new addition to the list of insect-pollinators of Ber already reported.

It is concluded that *P. dorsalis* may not be considered as a nuisance but an important pollinator of the Ber and its nests near or in the Ber orchards should not be destroyed.

College of Agriculture, J. S. DHALIWAL,  
Punjab Agricultural Univ.,  
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## REVIEWS AND NOTICES OF BOOKS

**Gene and the Genetic Code: The Chemical Basis of Life.** By J. D. Cherayil. (Tata McGraw-Hill Publishing Company New Delhi-49), 1974. Pp. 187. Price Rs. 24-00.

Although Watson and Crick postulated the double helical structure for the genetic material DNA in 1953, this discovery did not have the immediate impact it should have. With the recognition of this work by the award of Nobel Prize (for Physiology and Medicine) in 1962, there was a sudden explosion in knowledge of the chemical basis of genetics and a new branch of science, molecular biology, came into existence. In less than 5-10 years the finer details of the genetic code, protein biosynthesis, etc., were worked.

In this book of 187 pages Dr. Cherayil tells us the story of these exciting developments. The book is divided into 8 Chapters—Introduction; Concept of the Gene; Gene and Protein; Chemical Nature of the Gene, Nucleic Acids and Proteins; Deciphering the Genetic Code; Mechanism of Nucleic Acid Biosynthesis; Mechanism of Protein Biosynthesis; Other Functions of the Gene. Each Chapter is self-contained with its own bibliography.

The book is written in a simple and lucid style. The concepts are stated clearly. It is also happily free from many printing and grammatical errors; the reviewer found only one or two printing errors and a single grammatical error—more simpler—on p. 47.

The effort of the author to pack as much information as possible into this little book has resulted in a scanty treatment of certain topics. Chapter 5 on deciphering the genetic code in some places sounds like a dictionary of scientific words in molecular biology. This has also lead to juxtaposition of certain statements which do not follow a logical order. Further, in his effort to explain certain concepts in clear terms, he has repeatedly used the phrase "in other words" which has a jarring effect. There are a few loose statements such as the one on p. 27 on the net charge on a protein molecule and the one on p. 57 on the secondary structure of a protein.

The book covers adequately all the aspects of the gene and the genetic code. However, the reviewer wishes that a chapter on genetic engineering, at least on the possibilities and limitations, had been added;

the author makes a timely mention of this on p. 127. The problems in this area are highly exciting and at the same time it has raised moral and ethical questions.

The book is highly readable and will certainly be useful to those who want to mount students in modern sciences and in other fields like Chemistry and Physics. This can be an excellent popular science book which the reviewer wishes that such books were available in Indian languages for the benefit of the non-English-knowing public.

M. S. N.

**Drug Development Communications** (Vol. 1, No. 1, Editors: Christopher T. Rhodes, Marcel Dekker, Inc., 270 Madison Avenue, New York, N.Y. 10017, Pp. 87).

This new journal supplements and complements the information provided by the many existing pharmaceutical journals.

The article on "Modern pharmaceutical development" analyses and discusses the role of development in pharmaceutical industry in terms of products and processes with examples of the organizational form of development groups in four technologically advanced countries.

How the workers' morale can be considerably improved by the assistance provided by a computer is very well depicted in the article "The people computer interface in a capsule molding operation".

Other contributions include "Some physical characteristics of microcrystalline Cellulose", "Perfluorooctyl bromide emulsions as radio opaque media" and "A kinetic study of the solid state transformation of sodium bicarbonate to sodium carbonate".

The journal intends to cover all aspects of the development and production of drugs including both the technical and organizational aspects of the pharmaceutical industry.

M. SIRSI.

**Rothamsted Experimental Station Report for 1973**, Parts I and II, (Lawes Agricultural Trust, Harpenden, UK, 1974, Part I: Pp. 412; Part II: Pp. 275, Price both parts £3.00).

The progress of work at Rothamsted continues to be as stimulating as ever. This year a new Department of Molecular Structures has been added to the station. Significant findings reported are the methods attempted saving nitrogen loss, use of adhesive and microencapsulation in the formulation of pesticides in regulating their selectivity and persistence.

Control of viral diseases of plants has always been a great problem, the methods adopted at

present being mainly vector control, etc. The station finding that injection of polyacrylic acid gave control of TMV is reassuring and gives hope that a spray reagent may be found.

Part II which consists of special papers by the staff of the station. The papers on physiology of grain yield and root growth of cereals are particularly interesting.

V. N. V.

**Treatment of Inborn Errors of Metabolism.**

*Current Treatment and Future Prospects.* By J. W. T. Seakins, R. A. Saunders and C. Toothill. (Churchill Livingstone, Edinburgh), 1973. Pp. 260. Price £7.00.

Inborn errors of metabolism constitute a fascinating spectrum of diseases which result from congenital deficiency of particular enzymes caused by the presence of an abnormal or mutant gene. After identification of the diverse biochemical abnormalities in these diseases, the present lines of research have been aimed at the modification or alleviation of these defects by appropriate therapeutic measures. The status of current treatment as well as future prospects form the subject of this monograph which reports the proceedings of the tenth symposium of the Society for the Study of Inborn Errors of Metabolism held in United Kingdom in 1973.

The initial chapters deal with the assessment of dietary treatment of Phenylketonuria (PKU). The results of the collaborative study conducted in U.S.A. from 1967 have been optimistic. The development of the treated PKU children has been found to be normal, irrespective of the mild or moderate levels of phenylalanine in their diets. The hyperphenylalaninaemic variants and the reasons for the low incidence of PKU in females have also been discussed. The important questions as to when to wean these children from the special diet and the probable consequences with regard to growth of intelligence have been analysed. The discussions of the dietary treatment of Histidinaemia, Prolinaemia, Hydroxyprolinaemia and Galactosaemia reflect the particular problems pertaining to these conditions.

Professor Scriver in his Milner lecture has reviewed the several aspects of the hereditary vitamin dependencies or the vitamin responsive inborn errors of metabolism. A perusal of the list (Table 3, p. 138) indicates the universality of the several organ systems involved in these conditions as well as the identified apoenzymes which have been proven or presumed to be the aetiological factors. The discussion includes the guidelines for future research as well as the hazards which follow the indiscriminate use of certain vitamins. The

other papers deal with the treatment of vitamin D resistant rickets as well as the possibilities of alteration at the molecular level by therapeutic measures aiming at the prevention of accumulation of toxic compounds.

Treatment of hyperbilirubinaemia and mucopolysaccharidoses as well as the metabolic aspects of leucodystrophies and lipidoses form the themes of the other chapters.

This elegant monograph contains a wealth of information and will prove of great value to paediatricians, biochemists, neurologists and all others interested in diseases of metabolism.

A. G. KRISHNA.

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**Algae : Form and Function.** By G. S. Venkatraman, S. K. Goyal, B. D. Kaushik and Paromita Roy Choudhury. (Today and Tomorrow's Printers and Publishers, New Delhi), 1974. Pp. i-v + 562. Price Rs. 75.00 or \$ 15.00.

Generally, the algae have been taught in the various Universities in India since quite a long time, purely from the point of their structure, life-history, interrelationships and their bearing on the origin of land plants. This has mainly been due to the strong influence of the classical work of Dr. F. E. Fritsch in his *magnum opus* entitled *The Structure and Reproduction in Algae* published in two volumes (1935, 1942). The recent advances in the various disciplines of Botany particularly from the experimental angle which mostly includes cultural studies and the study of plant cells at the ultra level have thrown significant light for a proper understanding of their structure and metabolism. From these points of view, the algae have served as suitable material for providing a rich source of information. Taking all these points into consideration, Dr. Venkatraman and his co-authors have made a creditable attempt in a novel manner by providing an account of the algae from the point of view of their form and function. After an interesting foreword by Dr. G. E. Fogg and a good preface by the authors on the scope of the work, there are extremely well-written accounts on General Morphology and Ecology, Metabolism and Economic Aspects of Algae.

The entire approach is new because the information provided is organised into a series of well-thought-out questions with cogent answers on the structure at the ultra level, metabolism and physiology and economic uses of algae.

The accounts on cell organization, physiology and genetics are particularly well dealt with, focussing at the same time the most recent discoveries.

After reading the book, such aspects as general metabolism relating to nutritional requirements, respiration, nitrogen fixation, amino acids and proteins, and mutation and genetics, one wonders at the great potentialities the algae can provide for future work. One is also bewildered at the great economic uses to which they can be employed.

While most of the illustrations are well drawn the photomicrographs could have been better if they were printed on suitable art paper. At the end of each chapter there is an up-to-date bibliography.

On the whole Dr. Venkatraman, who is well known for his expert knowledge on this subject and his co-authors, have to be congratulated for bringing out this interesting treatise on algae from a thoroughly modern approach and their desire that it should be useful to the student community by dedicating the book to them is most apt. This book must find a place in the libraries of all educational and research institutions.

K. SUBRAMANYAM.

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#### ANNOUNCEMENTS

##### Winter School on Physiological Fluid Dynamics

The Winter School, sponsored by the Indian National Science Academy, New Delhi, will be held at Indian Institute of Technology, New Delhi, from December 8-19, 1975. The travel and local expenses of the participants whose papers are accepted for presentation will be met by the organizing committee. Those interested in contributing the Research papers are requested to send the abstract of the papers by July 31, 1975.

For further details and information, please write to : Prof. M. P. Singh, Department of Mathematics, Indian Institute of Technology, New Delhi 110 029.



## INFORMATION TO CONTRIBUTORS

CURRENT SCIENCE is the Premier Science Fortnightly of India published by the Current Science Association, Bangalore and issued on the 5th and 20th of each month. All material intended for publication in CURRENT SCIENCE and books for review should be addressed to the Editor, *Current Science*, Raman Research Institute, Bangalore-560006.

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References are indicated by superscripts in the text. The style of references should be *Journal*: The names of the authors, the journal, year, volume and page.

*Book*: Authors names, the title of the book, name and location of the publishers, year and page.

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# TOTAL ELECTRON CONTENT OF THE IONOSPHERE OVER THE MAGNETIC EQUATOR\*

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## ABSTRACT

Total electron content of the ionosphere ( $N_T$  at Kodaikanal (dip  $3.4^\circ$  N, Geogr. longitude  $77^\circ$  E), is measured using the faraday rotation of the 40 MHz signals from the low orbiting beacon satellites explorer 22 and 27 during the period November 1964 to December 1966. The diurnal variation of  $N_T$  is compared with the diurnal variation of the maximum electron density in the F region,  $N_m$ . The noon bite-out which is present in  $N_m$  is not seen in  $N_T$ . The latitudinal variation of  $N_T$ , obtained by combining the observations at Kodaikanal and Ahmedabad indicate the presence of the equatorial anomaly in  $N_T$  with peak around  $15^\circ$  dip latitude. The results are discussed in relation to the observations at other equatorial stations in African and American sectors. The latitudinal anomaly in neutral density is suggested to play a role in the latitudinal variation of  $N_T$ .

## INTRODUCTION

THE equatorial ionosphere is characterised by the well-known  $F_2$  anomaly, i.e., the latitudinal variation of the midday value of  $f_0F_2$  shows a bite-out at the dip equator and the daily variation of  $f_0F_2$  at equatorial station shows a bite-out around noon. The wide network of the ionospheric sounding stations has provided understanding of both the latitudinal as well as the diurnal anomaly of the  $F_2$  region under different geophysical conditions. The radio beacons on-board satellites have provided a useful method of computing total columnar electron content in the ionosphere (TEC). The measurements of TEC at Huancayo by Blumle (1962), and at Ibadan by Olatunji (1967), showed no noon bite-out. Rufenach *et al.* (1968) analysing faraday rotation records of the 54 MHz signals from the Transil 4-A satellite at Bangkok showed that TEC increased monotonically from the morning till noon or early afternoon hours while the peak electron density ( $N_mF_2$ ) showed the midday dip and late afternoon maximum (for the low sunspot considered). Combining the observations at Hong Kong, Bangkok and Singapore, they found that latitudinal variation of TEC during the daytime showed distinct trough over the equator with peaks at about  $\pm 30^\circ$  magnetic dip. Rastogi *et al.* (1973), combining the observations at Thumba and Ahmedabad, showed distinct development of the latitudinal anomaly in TEC with time after 06 hr and its vanishing after 20 hr, but no diurnal anomaly

in TEC was evident over Thumba. It may be mentioned that other authors have suggested the midday bite-out to be present in TEC at equatorial stations, viz. Skinner (1966) for Zaria (dip  $2^\circ$  N), Yeboah Amankwah and Koster (1972) for Legon (dip  $9.4^\circ$  N). However, Bandyopadhyay (1970) showed a dip in TEC at Huancayo at about 14 hr and Onwukwe (1974) found a dip in TEC at Ibadan around 15 hr. With these varied data of TEC at equatorial stations, it was felt necessary to examine the faraday rotation observations at Kodaikanal (dip  $3.4^\circ$  N) for the low sunspot years 1964-66.

## DATA AND RESULTS

The recording equipment consisted of E-W oriented dipoles fed to Hammerlund communication receivers through a HF converter and the output was recorded by strip chart pen recorder. Only 40 MHz records are analyzed for the present study. Total electron contents were calculated for every minute during the pass using the total rotation method ( $\Omega = K M N_T f^2$ ) where the symbols have the usual meanings. Near the QT region the differential formula ( $d\Omega = K N_T dM/f^2$  over short interval of time) was used. Regular ionospheric sounding records are obtained at Kodaikanal every fifteen minutes. The ionogram taken at time nearest to the time of the satellite transit at Kodaikanal was analyzed for the electron density profile using Budden's matrix method (1954) and therefore from the height of peak  $F_2$  ionization ( $h_mF_2$ ) and the peak electron density ( $N_mF_2$ ) and semi-thickness ( $Y_mF_2$ ) of the  $F_2$  region were computed. Horizontal drift velocity ( $V_k$ ) at ionospheric  $F_2$  region was measured at Thumba using spaced receiver technique

\* Part of this paper was presented at the Symposium on Beacon Satellite Investigations of the Ionosphere Structure and ATS-F Data held at Moscow in November, 1974.

March 1971, and these data are also used for comparison with other data here.

Figure 1 shows the average diurnal variation over the period 1964-66 of the parameters  $N_T$ ,  $N_m F_2$ ,  $h_m F_2$ ,  $Y_m F_2$  and  $V_h$  at Thumba. The number of observations of  $N_T$  measurements for any particular hour are also indicated at the top of the diagram. The observations around midnight are very few but for the daytime hours there are sufficient observations for any of the hours.

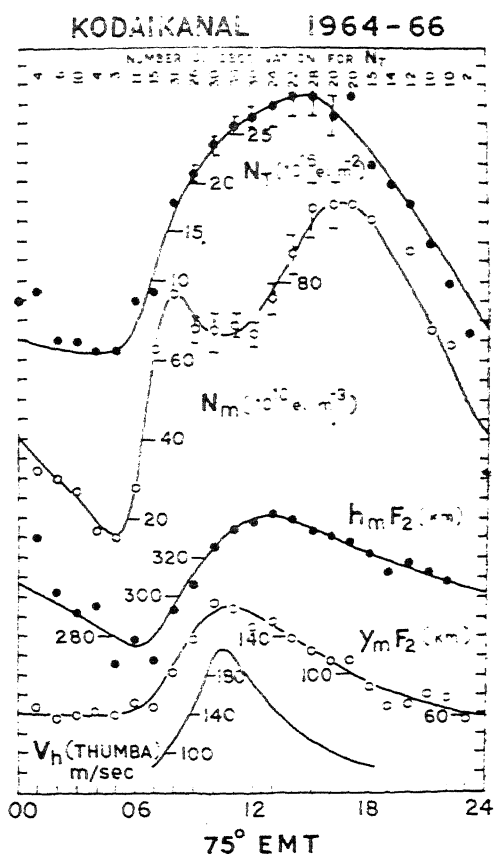


FIG. 1. Diurnal variation of total electron content ( $N_T$ ), peak electron density in  $F_2$  region ( $N_m F_2$ ), height of peak  $F_2$  ionization ( $h_m F_2$ ) and semithickness  $Y_m F_2$  of the ionosphere at Kodaikanal. Also shown is the  $F$  region horizontal drift velocity,  $V_h$ , at Thumba. Note that the maxima in  $V_h$ ,  $Y_m F_2$ , and  $h_m F_2$ , and the minimum in  $N_m$  coincide fairly well in time.

The mean value of  $N_T$  shows a minimum value around 04 hr, increases sharply after sunrise reaching a peak around 15 hr, thereafter decreasing steadily till 04 hr. The diurnal ratio of  $N_T$  is about 10. There is no evidence of the midday bite-out in  $N_T$ . The  $N_m$  curve shows minimum value around 05 hr,

and two maxima at 08 hr and around 16-17 hr with a bite-out around 11 hr. the diurnal ratio is about 6. The height of peak ionization  $h_m F_2$  is minimum around 06 hr and maximum around 13 hr. The semithickness  $Y_m F_2$  is maximum around 11 hr. The  $F$  region ionospheric drift velocity  $V_h$  is also maximum around 11 hr. The maxima of  $V_h$ ,  $Y_m$  and minimum of  $N_m F_2$  show clearly that the decrease of  $N_m F_2$  is due to the electrodynamic uplift of the ionization over the magnetic equator during the midday hours.

In Fig. 2 are shown the diurnal variation of  $N_T$  at the equatorial station Kodaikanal (dip  $3^\circ N$ ) and at tropical peak ionization region, Ahmedabad (dip  $34^\circ N$ ) for comparison. The diurnal variation of  $N_T$  is very similar at both the stations the maximum being around 14-15 hr. local. However, the values of  $N_T$  are reasonably larger at Ahmedabad than at Kodaikanal between 11 and 16 hr.

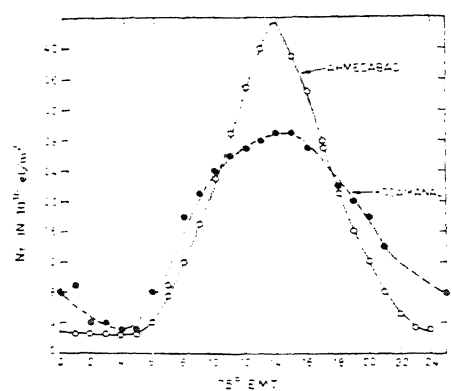


FIG. 2. Comparison of diurnal variation of  $N_T$  at Kodaikanal (closed circles and broken line) with that at Ahmedabad (open circles and continuous line), indicating the depletion of ionization at Kodaikanal around noon relative to Ahmedabad.

From a particular station the satellite goes through about  $15^\circ$  latitude during the useful recording period and so by combining the results of the same passes at the two stations one can get the latitudinal variation of  $N_T$  from the geographic equator to  $30^\circ N$  latitude. The latitudinal variation of TEC derived from some such satellite passes common to Kodaikanal and Ahmedabad are shown in Fig. 3. We have chosen the passes around midday hours only. It is clearly seen that the value of TEC is minimum around the dip equator and is maximum between  $15^\circ$  and  $20^\circ$  dip latitude, very similar to the latitudinal behaviour of  $N_m F_2$ .

Thus it is confirmed that at least in the Indian zone during low sunspot years the  $N_T$  does show the latitudinal anomaly but the diurnal anomaly is not evident over the magnetic equator.

The diurnal as well as the latitudinal anomaly in  $N_T F_2$  is supposed to be the consequence of the so-called "fountain effect": the ionization over the magnetic equator is lifted up by the  $\vec{E} \times \vec{B}$  force during the daytime and later the ionization diffuses to tropical latitudes along the magnetic lines of force. If it is wholly true then the daily and latitudinal variations of both  $N_T$  and  $N_m$  should be very similar to each other. The absence of the daily anomaly of  $N_T$  at the equator but the presence of latitudinal anomaly of  $N_T$  during midday hours suggests some modifications of the classical theory. First the excess ionization around dip  $\pm 30^\circ$  may be due to the accumulation of ionization over a large volume of the equatorial ionosphere: at a particular station in the equatorial zone the transport of ionization away from the region is too small to produce a midday bite-out in  $N_T$ .

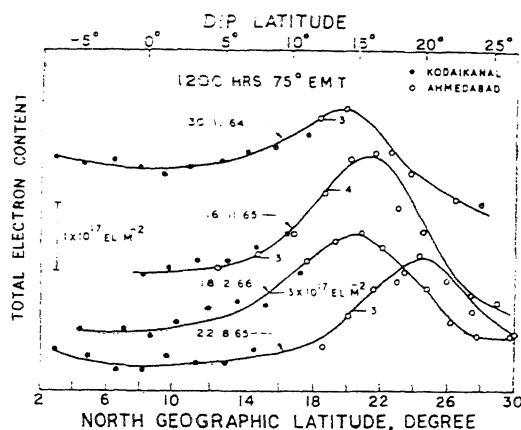


FIG. 3. A few cases of latitudinal variation of  $N_T$  combining the observations at Kodaikanal (closed circles) and Ahmedabad (open circles) clearly showing the equatorial anomaly in  $N_T$ .

Recent observations have indicated the presence of latitudinal anomaly in the neutral composition 20% higher for  $N_2$  and 10% higher for O at  $\pm 20^\circ$  magnetic latitudes than at equator (Hedin and Mayr, 1973; Newton and Pelz, 1969; Anderson, 1966). Chandra and Goldberg (1964) had theoretically indicated the geomagnetic control of neutral density in the lower F-region when the ion-neutral interaction is dominant. This brings to question if the ionization anomaly is wholly due to the transport

and diffusion of ionization or the neutral anomaly has a major share in the equatorial anomaly of the ionization. It is also felt that the daily variation of  $N_T$  at the equator may have large day-to-day and seasonal variation such that the daytime bite-out in  $N_T$  is not evident on averaging. The measurement of  $N_T$  at a number of equatorial stations using the beacon signals from a geostationary satellite would greatly help solving the problem of the equatorial anomaly. The forthcoming positioning of the ATS-6 satellite at  $35^\circ$  east longitude would provide a unique opportunity to Indian scientists. It may be mentioned that the recording of the Faraday rotation of 137 MHz beacon signals from Syncom III Satellite at Trichy (dip  $4-8^\circ$  N) have shown to us that this method is not very useful at these latitudes (Deshpande, 1975—private communication). It is therefore stressed that the measurements of  $N_T$  at low latitude should be done using group delay method rather than by the Faraday rotation method.

#### ACKNOWLEDGEMENTS

The authors wish to thank Prof. K. R. Ramathanan for his keen interest in this project. Financial support from the Department of Space, Government of India, is gratefully acknowledged.

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# SPECTROPHOTOMETRIC DETERMINATION OF SOME ORGANIC NITROGEN BASES AND IODINE IN DICHLOROETHANE

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## ABSTRACT

A new, rapid and simple spectrophotometric method for the determination of brucine, strychnine, 1, 10-phenanthroline, 2, 2'-bipyridyl and iodine has been developed based on the formation of triiodide ion *via* the charge transfer complex formation. Dichloroethane is found to be the most suitable solvent.

## INTRODUCTION

**S**PECTROPHOTOMETRIC determination of a number of compounds (donors and acceptors forming charge transfer complexes) is based on the charge transfer band maxima of the complexes<sup>1-4</sup>. Some methods reported for the estimation of the bases are generally based on their basic<sup>5-8</sup> and redox<sup>5-7</sup>, properties and on spectral characteristics<sup>9</sup> of these donors.

Brucine and strychnine have been estimated titrimetrically<sup>5</sup> involving the formation of relatively insoluble compounds with bichromate, mercury (II) and thiocyanate, the method being completed by titrating the excess reagent suitably. These procedures obviously are time consuming; 1, 10-phenanthroline and 2, 2'-bipyridyl are determined<sup>5</sup> analogously, based on the formation of the insoluble silver(II) salts. The titrimetric<sup>10</sup> determination of 1, 10-phenanthroline, 2, 2'-bipyridyl, brucine and strychnine in 50% acetic acid-acetic anhydride solvent, potentiometrically with perchloric acid, indicates that these are monoacidic bases.

We have earlier reported<sup>11</sup> a spot test for the detection of 1, 10-phenanthroline, iodine and iodate, based on the formation of a complex between the base and iodine. However, there seems to have been no report of the determination of these nitrogen bases, utilizing the ultimate formation of triiodide ion. The present paper describes the spectrophotometric determination of these bases with iodine *via* the inner complex formation. Incidentally it is possible to utilize the procedure for the determination of iodine also. The method is quite simple and rapid as compared to earlier methods.

## EXPERIMENTAL

**Reagents.**—1, 10-phenanthroline (E. Merck) was dried at 105° C and recrystallised from benzene. (m.p. 117° C). 2, 2'-Bipyridyl (E. Merck) was recrystallised from benzene. Iodine (B.D.H. India A.R.) was purified by resublimation under reduced pressure. Chloroform, 1, 2-dichloroethane, methanol were used after purification by distillation by standard methods<sup>12</sup>. Brucine and strychnine

(chempure, Analar) were recrystallised from chloroform.

## PROCEDURE

**Spectra.**—Absorption measurements were made with Beckmann DU Spectrophotometer at room temperature (25° C) using matched silica cells, 1 cm pathlength. The spectra of the mixtures of brucine-iodine, 1, 10-phenanthroline-iodine, 2, 2'-bipyridyl-iodine, strychnine-iodine in the solvent were scanned with iodine in the solvent as blank. A characteristic new band at about 365 nm indicates the formation of triiodide ion, *i.e.*, inner complex formation. The colour of the solution at this stage is yellow.

**Procedure for the nitrogen bases.**—Suitable aliquots of the nitrogen bases from the stock solutions, prepared by dissolving accurately weighed samples in the solvent, are transferred to 10 ml volumetric flask. Iodine solution (1 ml) is added to each flask and the contents made up to the mark with the solvent. The absorbance of the solution are measured at the 365 nm against the reagent blank. A calibration curve is drawn from the results. The sample solution is then treated with an excess of the reagent and its absorbance measured against the reagent blank. The amount of the base is then read off from the calibration curve.

TABLE I  
Titrimetric determination of mole ratio

| Volume of 1,10-phenanthroline (O.P.) | Volume of iodine | Amount of O.P. (gm) | Amount of iodine (gm) | Mole ratio |
|--------------------------------------|------------------|---------------------|-----------------------|------------|
| 10 ml.                               | 7.8 ml.          | 0.2507              | 0.4705                | 1.86       |
| 10 ml.                               | 8.1 ml.          | 0.2507              | 0.4887                | 1.95       |
| 10 ml.                               | 3.1 ml.          | 0.2493              | 0.5260                | 2.08       |
| 10 ml.                               | 3.2 ml.          | 0.2493              | 0.5420                | 2.15       |

**Procedure for iodine.**—Suitable aliquots of iodine from the stock solutions, prepared by dissolving weighed samples in chloroform are transferred

TABLE II  
*Spectrophotometric determination of nitrogen bases and iodine*

| Solvent              | Reagent    | Constituent determined | Amount of constituent ( $\mu\text{g/ml}$ ) |       |
|----------------------|------------|------------------------|--|-------|
|                      |            |                        | Taken                                      | Found |
| Chloroform           | Brucine    | Iodine                 | 2.00                                       | 2.00  |
|                      |            |                        | 5.00                                       | 5.02  |
|                      |            |                        | 10.00                                      | 9.95  |
|                      |            |                        | 20.00                                      | 19.90 |
| Chloroform           | Iodine     | Strychnine             | 5.00                                       | 5.00  |
|                      |            |                        | 10.00                                      | 10.10 |
|                      |            |                        | 20.00                                      | 19.95 |
|                      |            |                        | 30.00                                      | 29.50 |
| Chloroform           | Iodine     | Brucine                | 0.60                                       | 0.60  |
|                      |            |                        | 1.50                                       | 1.51  |
|                      |            |                        | 3.00                                       | 2.98  |
|                      |            |                        | 6.00                                       | 5.98  |
|                      |            |                        | 15.00                                      | 14.95 |
| Chloroform           | Strychnine | Iodine                 | 10.00                                      | 10.00 |
|                      |            |                        | 20.00                                      | 20.20 |
|                      |            |                        | 40.00                                      | 39.50 |
| 1, 2, Dichloroethane | Iodine     | Brucine                | 0.40                                       | 0.40  |
|                      |            |                        | 0.80                                       | 0.805 |
|                      |            |                        | 2.40                                       | 2.42  |
|                      |            |                        | 3.60                                       | 3.55  |
| 1, 2, Dichloroethane | Brucine    | Iodine                 | 0.90                                       | 0.90  |
|                      |            |                        | 2.60                                       | 2.62  |
|                      |            |                        | 8.50                                       | 8.40  |
|                      |            |                        | 22.00                                      | 21.55 |
| 1, 2, Dichloroethane | Iodine     | 1, 10-phenanthroline   | 155  | 156   |
|                      |            |                        | 225  | 223   |
|                      |            |                        | 340  | 344   |
|                      |            |                        | 455  | 449   |
| 1, 2, Dichloroethane | Iodine     | 2, 2' Bipyridyl        | 320  | 322   |
|                      |            |                        | 450  | 455   |
|                      |            |                        | 578  | 572   |
|                      |            |                        | 650  | 645   |
| 1, 2, Dichloroethane | Iodine     | Strychnine             | 2.00                                       | 2.00  |
|                      |            |                        | 4.50                                       | 4.45  |
|                      |            |                        | 12.00                                      | 11.90 |
|                      |            |                        | 18.50                                      | 18.40 |
| Methanol             | Iodine     | Strychnine             | 9.50                                       | 9.80  |
|                      |            |                        | 2.40                                       | 2.55  |
|                      |            |                        | 10.50                                      | 10.80 |
|                      |            |                        | 20.00                                      | 20.50 |
| Methanol             | Iodine     | Brucine                | 1.50                                       | 1.60  |
|                      |            |                        | 3.00                                       | 3.10  |
|                      |            |                        | 9.50                                       | 9.80  |
| Methanol             | Iodine     | 1, 10-phenanthroline   | 60.0                                       | 61.5  |
|                      |            |                        | 90.0                                       | 92.5  |
|                      |            |                        | 125.0                                      | 120.0 |
|                      |            |                        | 215.0                                      | 220.0 |
| Methanol             | Iodine     | 2, 2' Bipyridyl        | 120.0                                      | 124.0 |
|                      |            |                        | 224.0                                      | 229.0 |
|                      |            |                        | 340.0                                      | 348.0 |
|                      |            |                        | 450.0                                      | 460.0 |



10 ml volumetric flasks. One millilitre of brucine solution (excess) is added to each flask and the contents made up to the mark with the solvent. The absorbance of the solutions are measured at 365 nm against the reagent blank. The concentration of the unknown solution is calculated by referring the absorbance data to the calibration curve.

### RESULTS AND DISCUSSION

In our previous communication<sup>11</sup>, we have reported the formation of a blue precipitate which slowly changes its colour to brown in course of time. Further it has also been observed that the absorbance of a solution of 1, 10-phenanthroline and iodine in chloroform at 365 nm increases. This is probably due to the transformation of an outer complex to inner complex.

Ashworth *et al.*<sup>13</sup> studied the polarographic end point method for the titration of organic nitrogen bases, with iodine. They also found that some precipitate first forms which then takes on some more iodine to form an iodine richer product but could not explain the widely fluctuating data. The gravimetric method, as also the titrimetric method (of back titrating the excess iodine in the filtrate) in this investigation showed that the stoichiometry of 1, 10-phenanthroline : iodine = 1 : 2 (approx.) as can be seen from the results in Table I. Our results on the gravimetric and titrimetric methods are exactly similar to those of Ashworth *et al.*<sup>13</sup>.

However, such difficulties in the spectrophotometric determination of these bases do not seem to arise with 1, 2-dichloroethane as the solvent (Table II). The deviations observed in the gravimetric or titrimetric method may be due to slow transformation of the outer complexes, first formed to inner complexes. With comparatively stronger bases like brucine and strychnine the formation of the inner-complex and consequently the trihalide ion may be fast enough, so that the determination of these bases by the spectrophotometric method is not beset with such discrepancies. Similar type of fast transformations with the consequent immediate triiodide ion formation in more polar solvents has been reported earlier<sup>14-17</sup>.

The deviations observed in chloroform in the case of 1, 10-phenanthroline and 2, 2'-bipyridyl also can

be explained as due to the dependence of the transformation of the outer complex to inner complex and the dielectric constant. The variation to the extent of 5%, in the determination of these bases in methanol, may be due to the probable photo dissociation<sup>18-19</sup> of iodine-methanol complex.

### ACKNOWLEDGEMENTS

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# A STATIC UNIVERSE FILLED WITH SPINNING MATTER AND MAGNETIC FIELD

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**R**ECENTLY, the Einstein-Cartan theory has become a fashionable alternative to Einstein's original theory of gravitation<sup>1,3</sup>. Actually, it constitutes an extension of the latter theory, with the spin being incorporated from the beginning as a dynamical quantity. The spin angular momentum is related algebraically to the torsion tensor of the underlying manifold which no longer has a simple Riemannian structure. But, at the present observational level, the consequences of the ECT (Einstein-Cartan theory) are practically indistinguishable from those of general relativity, especially since the equations in empty space are identical in the two theories. The torsion of space-time does not vanish in regions filled with matter. A characteristic spin-spin repulsive interaction arises in the ECT which dominates the behaviour of matter at extremely high densities, say, above  $10^{54}$  g cm<sup>-3</sup>, and is able to prevent the occurrence of singularities in cosmology. Non-singular models of the Universe have been constructed explicitly<sup>4-7</sup>, which behave quite analogously to Friedmann-like models with a minimum non-zero radius of the Universe<sup>8</sup>. Trautman's original value of this minimum radius, ca. 1 cm, may be raised by ca. 13 orders of magnitude if the *f*-gravity is taken into account<sup>9</sup>. Then the maximum density of the Universe is only two or three orders of magnitude higher than the nuclear density, which sounds quite reasonable. Another approach to include the short range of the *f*-gravity (mediated by massive spin 2-mesons) through a reinterpretation of the cosmological constant<sup>10-12</sup> yields practically the same results: the possible prevention of the singularity. Instead of the spin-spin repulsive interaction characteristic for the ECT, there appears in the theory of Sivaram *et al.*<sup>12</sup> a repulsive scalar component of the massive *f*-gravity. The role of the cosmological constant in preventing the singularity is essential in the latter theory<sup>10-12</sup>. On the other hand, Einstein's static universe exists only with a nonvanishing cosmological constant. These two facts, together with the common consequence of the two approaches, makes it meaningful to ask for the possibility of a static cosmological model in the ECT. Such a model is only a very remote analogy of Einstein's static universe, because the presence of spins makes it anisotropic; numerical estimates yield values different from those of Einstein's model.

Our model is possible for the metric corresponding to Case 1 of Kantowski and Sachs<sup>13</sup> (a semi-closed model) :

$$ds^2 = -X^2(t) dx^2 - Y^2(t) [d\theta^2 + \sin^2 \theta d\psi^2] + dt^2 c^2 \quad (1)$$

when we assume that the metric functions *X* and *Y* do not depend on time. The cosmological substratum is a perfect fluid of energy density  $\rho$  and pressure *p*, with a density *S* of angular momentum, aligned along the *x*-axis. Also the magnetic field *H* points along this axis. The detailed equations are derived elsewhere<sup>14</sup>, and we give here only the final results for the two metric functions and the average radius *R* of the Universe :

$$X = \sqrt{\frac{(2-\gamma) 4\pi G}{\gamma} \frac{8\pi GHS_0}{c^5}}, \quad Y = \frac{c^2}{\sqrt{8\pi GH}},$$

$$R^3 = \sqrt{\frac{(2-\gamma) 4\pi G}{\gamma} \frac{S_0}{Hc}} \quad (2)$$

Here *c* is the light velocity, *G* — Newtonian gravitational constant, *S*<sub>0</sub> — total spin of aligned matter.  $\gamma$  is the coefficient ( $1 \leq \gamma < 2$ ) in the linear equation of state:  $p = (\gamma - 1)\rho$ . Energy density of the fluid and spin density may be expressed in terms of the magnetic field :

$$\rho = \frac{1}{2-\gamma} H^2, \quad S = \sqrt{\frac{\gamma}{(2-\gamma) 4\pi G}} c H \quad (3)$$

Let us assume that matter is composed of particles of mass *M* (identified with the nucleon mass) and spin  $\frac{1}{2} \hbar$ , and let *n* denote the numerical particle density while *N* is the total number of particles in a universe of radius *R*. Then we have:  $\rho = nMc^2$ ,  $S = \frac{1}{2} \hbar n$ ,  $S_0 = \frac{1}{2} \hbar N$ . Particle number density *n* and the magnetic field are well determined by the elementary constants :

$$n = \frac{\gamma}{\pi} \frac{Mc^4}{\hbar^2 G} \approx 0.6 \cdot 10^{79} \gamma \text{ particles per cm}^3,$$

$$H = \sqrt{\frac{(2-\gamma) \gamma}{4\pi G}} \frac{2Mc^3}{\hbar} \approx \sqrt{(2-\gamma)} \gamma \cdot 10^{38} \text{ Oe}.$$

The transverse radius *Y* does not depend on the amount of particles present, and is equal to  $Y = \hbar/2Mc \sqrt{2\gamma(2-\gamma)} \approx 0.7 \cdot 10^{-14} \text{ cm}$ .

$$\frac{1}{\sqrt{\gamma(2-\gamma)}},$$

while for a total of  $N = 10^{80}$  baryons we get the "longitudinal" dimension  $X = (2-\gamma) \cdot 0.3 \cdot 10^{30} \text{ cm}$ ,

and the average radius  $R = 2.5/\sqrt[3]{\gamma}$  cm. Both the longitudinal and transversal dimension may come close to each other for a very "stiff" equation of state, with  $\gamma$  approaching the value of 2. At the same time, magnetic field  $H$  goes to zero for  $\gamma \rightarrow 2$ . It is interesting to find that the "longitudinal" dimension of our universe goes linear with the total particle number  $N$ , while the "average radius" rises with  $N^{1/3}$ .

It is not possible to argue that the static model we present here has something to do with the actual universe in which we live. But in fact it may be related to the unstable initial stage of evolution of an expanding non-singular universe; such a stage might have lasted an indefinite amount of time before the instability resulted in expansion. Our model is classical, and it does not take into account the effects of pair creation, etc., which should occur at such high values of the magnetic field. In spite of all possible counterarguments against the reality of models of such kind, this may be an interesting result in itself, proving the possibility of deriving an anisotropic, static model with aligned spins and magnetic field in the framework of the ECT. No analogy to this in the general theory of relativity

can be found, apart from the remote case of the Einstein static universe, where the term with the cosmological constant plays a similar stabilizing role like our spin (or torsion)-induced term in the ECT.

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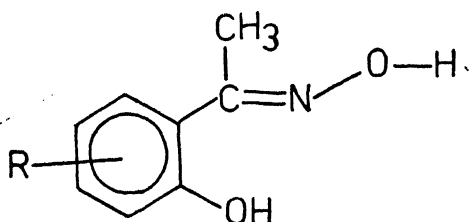
# OXOVANADIUM(IV) COMPLEXES WITH *o*-HYDROXY ACETOPHENONE OXIMES

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THE oximes<sup>1</sup> are mainly used in analytical chemistry as organic reagents to precipitate many metals. Potentiometric study<sup>2</sup> on *o*-hydroxy acetophenone oximes with oxovanadium(IV) has been reported in the literature. Saksena<sup>3</sup> *et al.*, have reported 2-OH-4 Me-5-Cl propiophenone oximes. Bielg and Mollinger<sup>4</sup> have prepared the salicylaldoxime complexes of vanadium (IV) and (V). They have isolated the vanadium (IV) complex with composition  $C_{14}H_{12}O_5N_2 V$  similar to ours.

The present series of investigations is to synthesize oxovanadium(IV) complexes with *o*-hydroxy acetophenone oximes and obtain structural information with the help of physicochemical methods. The oximes used are :



- R  
I. H  
II. 3.Me  
III. 4.Me  
IV. 5 Me  
V. 5.Cl

## EXPERIMENTAL

Vanadyl chloride was of BDH make. The substituted *o*-hydroxy acetophenones were prepared according to the standard methods<sup>5</sup>. Hydroxylamine hydrochloride was of reagent grade. The oximes were prepared by heating 1 g. of *o*-hydroxy acetophenone, 1 g. of  $NH_2OH \cdot HCl$  and 2 g. of sodium acetate in 10 ml. of ethanol on a steam bath for about an hour.

Vanadyl chloride (1 mole) in alcohol was treated with oximes (2 m mole) in the same solvent and refluxed for a while. The precipitated complex was filtered, washed with aq. alcohol and dried in vacuum over fused calcium chloride.

## ELEMENTAL ANALYSIS

The vanadium and nitrogen in the complexes were estimated by conventional methods as pentoxide and by Kjeldahl method. C and H were estimated by microanalytical Department of Osmania University, Hyderabad.

## RESULTS AND DISCUSSION

The analytical data given in Table I, shows that oxovanadium(IV) chloride forms complexes of 1 : 2 stoichiometry losing two of its chloride ions during the course of reaction. The molar conductance in DMF at  $10^{-3}$  M are too small to account for any dissociation of the complexes in that solvent. The magnetic moment at room temperature (Table I) closely agrees with the spin only value of oxovanadium(IV) complexes hitherto reported<sup>6</sup>. Bielg and Mollinger<sup>4</sup> reported a value of 1.70 B.M. for oxo-bis-(salicylaldoxime) vanadium(IV) complex.

## ELECTRONIC SPECTRA

The electronic spectra of the complexes were taken with a Beckman DK-2 Spectrophotometer in chloroform in the region 300–900 nm. The electronic spectra of oxovanadium(IV) complexes are discussed in the literature<sup>7</sup>, in terms of  $C_{4v}$  and  $C_{2v}$  symmetry. The lowering of symmetry from  $C_{4v}$  to  $C_{2v}$  has the effect of removing the degeneracy in the *d*-orbitals and thus four transitions are predicted in the above region.

In the present investigation the spectra are all of similar pattern indicating that they have the same geometry. Out of the two distinct bands, the band around 490 nm is assigned to  $d_{xy} - d_{x^2-y^2}$  transition. The broad intense maximum observed around 800 nm may be assigned to the  $d_{xy} - d_{yz}$ ,  $d_{xz}$  transition. We are less certain about the assignment of the third band in the high energy region which may be present as a tail of the charge transfer band.

## INFRARED SPECTRA

The infrared spectra were taken in KBr pellets on Perkin Elmer-337, in the range 4000–400  $cm^{-1}$  and the important frequencies are shown in Table I.

Comparison of the spectra of ligands with those of the complexes indicate bonding of oxygen of the

\* For correspondence.

TABLE I

Elemental analysis\*, magnetic moment and important IR frequencies\*\*

| Sl. No. | Complexes                                     | % V              | % N            | % C              | % H            | $\mu_{eff}$ at room temp. | $\nu$ (OH) inter- and intra-molecular H-bonded | $\nu$ (C=N)      | $\nu$ (C—O)      | $\nu$ (V=O) |
|---------|---|------------------|----------------|------------------|----------------|---------------------------|--|------------------|------------------|-------------|
| 1.      | VO (2-OH-Aceto-phenone Oxime) <sub>2</sub>    | 13.51<br>(13.90) | 7.71<br>(7.63) | 52.28<br>(52.60) | 4.58<br>(4.38) | 1.65                      | 3300s<br>(3340s<br>2650b)                      | 1548s<br>(1630s) | 1312s<br>(1285s) | 992s        |
| 2.      | VO (2-OH-3Me-Acetophenone Oxime) <sub>2</sub> | 13.12<br>(12.91) | 6.82<br>(7.08) | 55.22<br>(54.69) | 5.11<br>(5.06) | 1.60                      | 3290<br>(3340s<br>2650b)                       | 1550s<br>(1630s) | 1310s<br>(1290s) | 990s        |
| 3.      | VO (2-OH-4Me-Acetophenone Oxime) <sub>2</sub> | 12.90<br>(12.91) | 6.83<br>(7.08) | 55.51<br>(54.69) | 5.00<br>(5.06) | 1.72                      | 3300<br>(3330s<br>2650b)                       | 1550s<br>(1630s) | 1300s<br>(1285s) | 995s        |
| 4.      | VO (2-OH-5Me-Acetophenone Oxime) <sub>2</sub> | 12.46<br>(12.91) | 6.53<br>(7.08) | 55.46<br>(54.69) | 5.35<br>(5.06) | 1.65                      | 3170s<br>(3340s<br>2650b)                      | 1550s<br>(1635s) | 1300s<br>(1285s) | 988s        |
| 5.      | VO (2-OH-5Cl-Acetophenone Oxime) <sub>2</sub> | 11.54<br>(12.23) | 6.35<br>(6.72) | 43.98<br>(43.93) | 3.15<br>(3.02) | 1.57                      | 3220sb<br>(3325s<br>2650b)                     | 1545s<br>(1635s) | 1295s<br>(1285s) | 998s        |

\* The values in parentheses are calculated values.

\*\* The values in parentheses are for ligands.

OH group and nitrogen of the CH=N group<sup>8</sup>. Appearance of the intense band, in complexes around 980 cm<sup>-1</sup> indicates the monomeric nature of the complexes<sup>9</sup>.

All these observations indicate that these complexes have a coordination number of five. Generally five coordinate oxovanadium complexes have square pyramidal structure with oxygen at the apex.

## ACKNOWLEDGEMENT

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## LETTERS TO THE EDITOR

FRANCK-CONDON FACTORS AND  
r-CENTROIDS OF CF<sup>+</sup>

RECENTLY detailed rotational analysis of the A<sup>1</sup>Π — X<sup>1</sup>Σ<sup>+</sup> system of CF<sup>+</sup> molecule has been carried out by Verma<sup>1</sup> which is of considerable importance among the ionized molecular species. As Franck-Condon factors and *r*-centroids are useful in the determination of band strengths, the authors attempted to calculate the Franck-Condon factors and *r*-centroids of A<sup>1</sup>Π — X<sup>1</sup>Σ<sup>+</sup> system of CF<sup>+</sup> molecule.

The relative band strengths can be written as

$$P_{v', v''} = R_e^2 (\tilde{r}_{v', v''})^2 q_{v', v''}$$

where  $R_e$  is the electronic transition moment,  $\tilde{r}_{v', v''}$  is *r*-centroid of the *v'*—*v''* transition and  $q_{v', v''}$  denotes the Franck-Condon factor which controls the intensity distribution in a band system. The *r*-centroids of the A<sup>1</sup>Π — X<sup>1</sup>Σ<sup>+</sup> system of CF<sup>+</sup> molecule have been calculated by employing both graphical and quadratic methods<sup>2</sup> and are presented in Table I. The sequence difference  $\Delta\tilde{r} = \tilde{r}_{v'+1, v''+1} - \tilde{r}_{v', v''}$  is found to be approximately constant. It is of interest to note that  $r_{0,0}$  is slightly greater than the  $(r_{01} + \tilde{r}_{02})/2$  which suggests that the potentials are not very anharmonic.

TABLE I  
Franck-Condon factors and *r*-centroids of  
A<sup>1</sup>Π — X<sup>1</sup>Σ<sup>+</sup> system of CF<sup>+</sup> molecule

| <i>v'/v''</i> | 0     | 1     | 2     | 3     |
|---------------|-------|-------|-------|-------|
| 0. <i>a</i>   | 0.793 | 0.191 | 0.015 | 0.000 |
| <i>b</i>      | 1.252 | 1.352 | 1.475 | 1.637 |
| <i>c</i>      | 1.253 | 1.350 | ..    | ..    |
| 1. <i>a</i>   | 0.175 | 0.465 | 0.315 | 0.043 |
| <i>b</i>      | 1.177 | 1.262 | 1.361 | 1.484 |
| <i>c</i>      | 1.179 | 1.263 | 1.359 | ..    |
| 2. <i>a</i>   | 0.028 | 0.261 | 0.242 | 0.385 |
| <i>b</i>      | 1.112 | 1.186 | 1.271 | 1.370 |
| <i>c</i>      | 1.115 | 1.188 | 1.272 | ..    |
| 3. <i>a</i>   | 0.004 | 0.067 | 0.284 | 0.103 |
| <i>b</i>      | 1.055 | 1.121 | 1.196 | 1.280 |
| <i>c</i>      | 1.058 | 1.124 | 1.198 | 1.281 |

*a*—Franck-Condon factor; *b*, *c*—*r*-centroids by quadratic and graphical methods respectively.

Since  $|d\alpha/a|$  for A—X system of CF<sup>+</sup> molecule 7.5%, the Franck-Condon factors have been evaluated by the analytical method of Fraser and Jarman<sup>3</sup> with *r*<sub>0</sub>-shift correction and the results are presented in Table I along with the *r*-centroids.

Here the internuclear separation involves only a slight change ( $\Delta r_e = 0.04$  Å) in this transition and exhibits only a primary Condon parabola in the Franck-Condon factor array which can be seen in Table I. Also from Table I, we can definitely conclude that the bands beyond 3, 0 in the *v'*-progression and the bands beyond 0, 3 in *v''*-progression cannot be observed which is in accordance with the experimental observation.

Spectroscopy Laboratories,  
Andhra University, Waltair,  
India, January 31, 1975.

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D. V. K. RAO,  
P. T. RAO.

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MEASUREMENT OF K SHELL PHOTOELECTRIC  
CROSS SECTIONS FOR ELEMENTS IN THE  
RANGE Z = 42 TO Z = 74 AT 74.409 keV

OUR earlier measurements<sup>1-5</sup> of K and L shell photoelectric cross sections show that while there is a fairly good agreement between experiment and theory at high photon energies (well above K threshold), there seems to exist some discrepancy<sup>3-5</sup> at low energies (around K threshold). Even though the available experimental data at low energies are scanty, it is seen that the L shell cross sections are consistently higher than the theoretical values and the difference between experiment and theory increases as the photon energy decreases. To draw definite conclusions about K shell, more experimental data are needed<sup>5</sup> at low energies. We have measured the K shell cross sections at 74.409 keV in 11 elements 'Z' ranging from 42 to 74. The 74.409 keV photons were obtained from the K conversion of 279 KeV level in Ti<sup>203</sup>. The method of measurement was the same as that used in the previous measurements<sup>5</sup>. The results are compared with theory<sup>6-8</sup> in Table I.

It is seen that as in the previous measurements<sup>5</sup> at 36.818 keV, the present results also agree with theory within experimental errors but contrary to

TABLE I

Comparison of present measurements with  
existing theoretical  
calculations

- (1) — Scofield<sup>6</sup>.  
(2) — Schmickley-Pratt<sup>7</sup>.  
(3) — Rakavy-Ron<sup>8</sup>.

| Element | Z  | K shell photoelectric cross<br>sections at 74.409 keV |                                  |
|---------|----|---|----------------------------------|
|         |    | Present<br>measurements<br>b/atom                     | Existing values<br>b/atom        |
| Mo      | 42 | 254 ± 26  | (1) 295<br>(2) 295               |
| Ag      | 47 | 432 ± 42  | (1) 443<br>(2) 445               |
| Cd      | 48 | 440 ± 43  | (1) 480<br>(2) 480               |
| I       | 53 | 664 ± 66  | (1) 705<br>(2) 710               |
| Ba      | 56 | 863 ± 84  | (1) 865<br>(2) 850               |
| La      | 57 | 842 ± 82  | (1) 910<br>(2) 900               |
| Ce      | 58 | 937 ± 95  | (1) 975<br>(2) 970               |
| Sm      | 62 | 1269 ± 123  | (1) 1220<br>(2) 1220             |
| Gd      | 64 | 1417 ± 142  | (1) 1400<br>(2) 1390             |
| Er      | 68 | 1538 ± 157  | (1) 1650<br>(2) 1640             |
| W       | 74 | 2145 ± 219  | (1) 2220<br>(2) 2240<br>(3) 2220 |

previous observations all the experimental values are not lower than the theoretical values. It could therefore be inferred that in the previous measurements<sup>5</sup> all the experimental values came out to be lower than theoretical values accidentally. The present results also rule out the existence of any discrepancy for K shell similar to the one observed for L shell at low energies.

Department of Physics, K. L. ALLAWADHI.  
Punjab University, B. S. SOOD.  
Patiala-147002, January 27, 1975.

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### FLUOROMETRIC STUDY OF TETRABROMO-SODIUM FLUORESCIN

THE change in concentration of tetrabromo-sodium fluorescein (red dye) in aqueous solution causes a large shift in the excitation peak as compared to the emission peak. A systematic study of these shifts are reported in this note. A similar investigation has already been reported with fluorescein solution<sup>1</sup>. The dye concentration in the present studies is varied from  $10^{-7}$  M to  $10^{-4}$  M at room temperature ( $\sim 20^\circ$  C). With rise in molarity it is found that the excitation peak remains almost constant at 2970 Å ( $\sim 4.1$  eV) for the concentrations,  $10^{-7}$ – $10^{-5}$  M, but the peak position changes to 4660 Å ( $\sim 2.6$  eV) as the concentration is increased to  $\sim 10^{-4}$  M through  $\sim 5 \times 10^{-5}$  M. This large difference of 1.5 eV in the excitation peaks may be accounted for by assuming that the molecular form of the dye changes from monomeric to possibly dimeric or even polymeric at high concentration. However, the emission peak does not show a large change with change in concentration and the peak position shifts only from 5300 Å ( $\sim 2.3$  eV) to 5500 Å ( $\sim 2.2$  eV) as the concentration is changed from  $\sim 10^{-7}$  M to  $\sim 10^{-4}$  M. This may be taken to indicate that the monomeric form of the dye is also responsible for such emission at higher concentrations.

In the case of fluorescein<sup>1,2,3</sup> also, dimerisation takes place with increase of concentration. However, here both the excitation and emission peaks show a shift on dimerisation. There is no evidence of dissociation of dimers in the excited state, as is apparent in its tetrabromo derivative. It is known that the presence of bromine substituent in an aromatic compound usually leads to a reduction in fluorescence by encouraging intersystem crossing from the excited singlet to the triplet state. Also, for the tetrabromo derivative, the excitation and the fluorescence wavelength maxima are shifted to the longer wavelength indicating an increased free-

dom of the  $\pi$  electrons. Thus, for the excited dimer state of tetrabromo fluorescein, the transition singlet-triplet becomes more dominant as compared to singlet-singlet transition. Once the cross over to the triplet state is accomplished, the dimeric molecule in this metastable state may undergo dissociation into monomers through collisions with other excited dimeric molecules in the singlet state. The monomers resulting from dissociation then give rise to the observed fluorescence emission. If the viscosity of the solvent is increased or the temperature is lowered, the dissociation of the dimers would decrease and the fluorescence due to the dimers could be obtained<sup>4</sup>.

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#### FORCE CONSTANTS AND MEAN AMPLITUDES OF VIBRATION OF THE OXONIUM IONS

##### $\text{H}_3\text{O}^+$ AND $\text{D}_3\text{O}^+$

RECENTLY, Basile *et al.*<sup>1</sup> reported the spectra and normal coordinate analysis of the oxonium ions  $\text{H}_3\text{O}^+$  and  $\text{D}_3\text{O}^+$  assuming pyramidal structure. The general valence force field (GVFF) and Urey-Bradley force field (UBFF) were employed to compute the force constants. Stretching and bending diagonal force constants  $f_r$  and  $f_a$  were calculated from experimental frequencies of  $\text{H}_2\text{O}$  and  $\text{H}_3\text{O}^+$  using the relation of Nakamoto<sup>2</sup>. Unlike earlier workers, we report the GVFF constants without imposing any constraint as mentioned above. The mean amplitudes of vibration have also been computed.

Two sets of GVFF constants were computed using known procedures<sup>3</sup>. In the first set, five force constants were used:  $f_r$  (stretch),  $f_a$  (bend), the interaction constants  $f_{rr}$  (stretch-stretch),  $f_{aa}$  (bend-bend) and  $f_{ra}$  (stretch-bend). F and G matrices were taken from Nakamoto<sup>2</sup>. The two-dimensional secular equation in both the species were solved by Muller's L-matrix method<sup>4</sup>. The second set of four force constants were calculated ignoring the stretch-bend interaction constant  $f_{ra}$ . The

results thus obtained along with the previous values<sup>1</sup> are shown in Table I. The fundamental frequencies and molecular parameters of  $\text{H}_3\text{O}^+$  used in the present computation are:  $\nu_1(a_1) = 2700$ ,  $\nu_2(a_1) = 1125$ ,  $\nu_3(e) = 2600$ ,  $\nu_4(e) = 1665$ , O-H distance = 1.011 Å and H-O-H bond-angle = 110.4°. In order to test the reliability of the force constants, the fundamental frequencies of  $\text{D}_3\text{O}^+$  were calculated with the help of these constants which are reported in Table II.

TABLE I  
GVFF constants (in mdyne/Å) of  $\text{H}_3\text{O}^+$

| Method | $f_r$   | $f_{rr}$ | $f_a$ | $f_a$  | $f_{aa}$ |
|--------|---------|----------|-------|--------|----------|
| I set  | 3.888   | 0.181    | 0.019 | 0.638  | 0.027    |
| II set | 3.873   | 0.186    | 0.000 | 0.639  | 0.027    |
|        | (3.93)* | (0.21)   | ..    | (0.66) | (0.04)   |

\* Values in parentheses are from ref. 1.

TABLE II  
Comparison of observed and calculated fundamentals of  $\text{D}_3\text{O}^+$  (in  $\text{cm}^{-1}$ )

| $\nu_1$ | Observed Fundamentals | Calculated Fundamentals |           |
|---------|-----------------------|-------------------------|-----------|
|         |                       | Muller's method         | $f_a = 0$ |
| $\nu_1$ | 2000                  | 1927                    | 1929      |
| $\nu_2$ | 885                   | 848                     | 848       |
| $\nu_3$ | 1945                  | 1911                    | 1917      |
| $\nu_4$ | 1185                  | 1206                    | 1202      |

TABLE III  
Mean amplitudes of vibration (in Å) of  $\text{H}_3\text{O}^+$  and  $\text{D}_3\text{O}^+$

| Ion                    | Distance | Temperature |           |        |
|------------------------|----------|-------------|-----------|--------|
|                        |          | 0° K        | 298.15° K | 500° K |
| $\text{H}_3\text{O}^+$ | O-H      | 0.0822      | 0.0822    | 0.0822 |
|                        | H..H     | 0.1256      | 0.1256    | 0.1265 |
| $\text{D}_3\text{O}^+$ | O-D      | 0.0693      | 0.0693    | 0.0695 |
|                        | D..D     | 0.1043      | 0.1046    | 0.1059 |

The mean amplitudes of vibration were calculated following Cyvin's procedure<sup>5</sup>. The mean amplitudes for bonded and non-bonded distances at three



temperatures :  $T = 0^\circ \text{K}$ ,  $\bar{T} = 298.15^\circ \text{K}$  and  $T = 500^\circ \text{K}$  are shown in Table III.

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#### BRIGHTNESS WAVEFORMS OF ELECTROLUMINESCENCE FROM $(\text{ZnS}:\text{Cu})\text{H}$ DOUBLE-BAND LUMINOPHORS (UNDER CONCURRENT EXCITATION WITH ELECTRIC FIELD AND U.V. RADIATION)

WHILE the study of time-averaged electroluminescent brightness as a function of exciting field strength and frequency has been of immense help to understand the internal structure of electroluminescent solids, time-dependent studies in this context have been quite important in revealing various facts about the nature of trap levels in such systems. As a matter of fact, the trap levels in an electroluminescent solid play a vital role in the excitation of electroluminescence.

In the case of Cu-activated ZnS-type phosphors, the brightness waves generally repeat themselves with double the frequency of the applied sinusoidal electric field. These are also associated with secondary peaks either on ascending or on descending side of the primary peaks in certain cases<sup>1</sup>. Attempts have also been made by some workers to study the temperature dependence of these patterns in various species of phosphors. Patek<sup>2</sup> studied modifications introduced in these brightness waves, due to u.v. light in the presence of electric field. The effect of superimposed D.C. fields on these brightness waves were studied by Thornton<sup>3</sup>.

All these investigations were generally confined to the phosphors giving single-band emission. We give here an account of our findings as regards brightness-waves of emission under concurrent excitation with electric field and u.v. radiations in individual

bands in case of a  $(\text{ZnS}:\text{Cu})\text{H}$  double-band electrolumiphors<sup>4</sup>. Such studies are also helpful to understand the enhancement behaviour of the phosphor.

Permanent type of electroluminescent cells were used for these studies. The audio frequency electric signals were obtained by oscillator cum wide band amplifier unit. The EL cells were first excited by electric field and then simultaneously by electric field and u.v. radiations. The light signals so obtained were displayed on a double-beam CRO through an RCA-6217 photomultiplier tube. Appropriate interference filters were used to isolate the two emission bands.

The phosphor under study shows two peaks of electroluminescence, blue (4600 Å) and green (5200 Å) the relative intensity of which changes with the excitation frequency. The photoluminescence, on u.v. excitation, shows single peak of emission at 5200 Å. The time average study under concurrent excitation shows enhancement for blue peaks and quenching for green peaks<sup>5</sup>.

In the present case for blue transitions, no secondary peaks are observed; moreover the two primary peaks obtained corresponding to each half period of cycle are unsymmetrical and are joined together. The peak, corresponding to positive cycle, is observed to be smaller than that corresponding to -ve cycle. It is probably because of the two different electrodes of the cell, as the brightness waves do depend on the thermionic work function of the electrode in contact<sup>6</sup>. The joint action seems to be due to the delayed transitions caused by the trapping of electrons. However the green peaks give a more symmetrical picture. Here the secondary peaks are also observed on the ascending side of the primary peaks.

The green peaks appear to be not very sensitive to enhancement. They do not show any peculiar change on applying u.v. radiations (Fig. 1  $a_2$  and  $a_3$ ); only the level of peaks gets increased except at 100 c/s, where the level is decreased showing quenching effect (Fig. 1  $a_1$ ).

The enhancement effects, for the blue peaks are much pronounced. At 10 Kc/s both the peaks are simultaneously enhanced but the secondary peak associated with the -ve peak is suppressed (Fig. 1  $b_2$ ). The rise in level is about 25%. The results at 100 c/s are more interesting. For 22% u.v. radiation, the peak of -ve cycle is completely suppressed while the other, less in degree, shows the quenching effect (Fig. 1  $b_1$ ). As the u.v. intensity is further increased to 100%, the two sharp peaks are observed, the -ve one being quite small while the +ve one about ten times larger in magnitude

(Fig. 1  $b_3$ ). Upon applying u.v. radiations phase shifts are generally not observed.

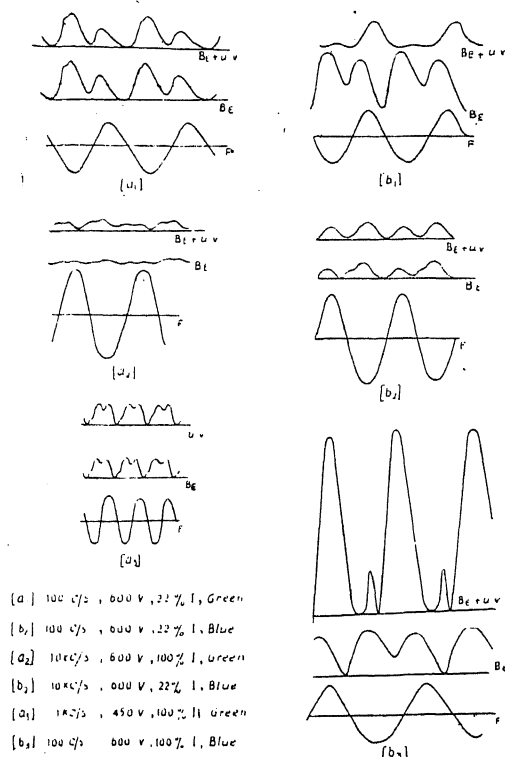


FIG. 1. The oscilloscopic study of brightness waves under concurrent action of electric field and u.v. radiation.

It has been seen that the enhancement or quenching depends on the order of overlapping of the two intensities, the more nearly equal the two intensities, the more the enhancement. The quenching for green peak seems to be due to too much of green photoluminescence.

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## MODIFIED FORMS OF HYDROUS ZIRCONIA

If excess of sodium carbonate is added with stirring to a solution of zirconyl chloride, a stable precipitate known commercially as carbonated hydrous zirconia is obtained. The exact nature of carbonated hydrous zirconia is not clear. Under certain conditions it conforms to the formula  $Zr_2O_3(OH)_2 \cdot CO_2 \cdot 7H_2O^{1,2}$ . For the present it may be viewed as a modified form of hydrous zirconia. The peculiar characteristics of carbonated hydrous zirconia prompted us to attempt the preparation of analogous modified oxides and to study their properties. The results are given below:

1. *Sulphited hydrous zirconia*.—Purified zirconyl chloride (10 gm) was dissolved in water (40 ml). Sodium sulphite solution (0.1 molar) was then added in drops with good stirring until no more precipitate was formed. The precipitate was then filtered, washed thoroughly with water and dried in the desiccator at room temperature. It was analysed to get the following results:

Found: Zr, 45.64%;  $SO_3$ , 19.70%.

$Zr_2O_3(OH)_2 \cdot SO_3 \cdot 4H_2O$  requires Zr, 45.55%;  $SO_3$ , 19.98%.

2. *Nitrited hydrous zirconia*.—The method of preparation was similar. Sodium nitrite solution was added in drops to a solution of the purified zirconyl chloride until the stable precipitate was obtained. It was filtered, washed with water and dried in desiccator. Analysis gave the following results:

Found: Zr, 40.81%;  $NO_2$ , 20.43%.

$ZrO(OH) \cdot NO_2 \cdot 3H_2O$  requires Zr, 40.69%;  $NO_2$ , 20.52%.

3. *Basic zirconyl chloride*.—Solid zirconyl chloride was mixed with sulphited hydrous zirconia or nitrited hydrous zirconia in the mole ratio of 1:1. On good mixing, clear solutions were formed because of the water of hydration originally present. No heating was required. From the filtered solution, gummy solids could be obtained either by adding excess acetone or by gentle evaporation *in vacuo*.

The results of analysis are given in Table I.

The molecular weights of 1 and 2 support the formulas  $Zr_2O_3Cl_2 \cdot 7H_2O$  and  $ZrO(OH)Cl \cdot 3H_2O$  respectively.

Carbonated, sulphited and nitrited hydrous zirconia show properties different from those of hydrous zirconia because of the influence of the extraneous ions contained by them. They are more amenable to dissolution in dilute acids than hydrous zirconia. Hydrochloric acid is able to displace these weak anions easily with chloride.

A dimeric structure can be postulated for sulphited hydrous zirconia, as in the case of carbonated hydrous

TABLE I

| Basic zirconyl chloride   | Precipitated by    |         |                        |         |
|---|--------------------|---------|------------------------|---------|
|   | (a) adding acetone |         | (b) gentle evaporation |         |
|   | Zr<br>%            | Cl<br>% | Zr<br>%                | Cl<br>% |
| (1) From sulphited hydrous zirconia and zirconyl chloride                                   | 42.4               | 16.5    | 42.5                   | 16.5    |
| (2) From nitrited hydrous zirconia and zirconyl chloride                                    | 42.5               | 16.6    | 42.5                   | 16.9    |
| $Zr_2O_3Cl_2 \cdot 7H_2O$ and $ZrO(OH)Cl \cdot 3H_2O$ : Both require Zr., 42.7% ; Cl, 16.6% |                    |         |                        |         |

zirconia. This is supported by its analytical results and by the molecular weight of the basic zirconyl chloride derived from it. On the other hand, nitrited hydrous zirconia seems to have a monomeric structure. Its analytical results and the molecular weight of the basic zirconyl chloride, derived from it, lend support to this view. The molecular weights in aqueous solution, by the F.P. method, varied with time due to slow ionization. In general the sulphited sample gave higher value (298) for the molecular weight than the nitrited sample (150).

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#### ***p*-NITROPHENYL ACID PHOSPHATASE ACTIVITY IN GERMINATING BARLEY SEEDS UNDER WATER STRESS**

WATER stress is known to impede protein synthesis<sup>1</sup>, enhance amino acid liberation<sup>2</sup> and change enzymatic<sup>3</sup> and polyribosome levels<sup>4</sup> in plants. However, the biochemical mechanisms responsible for water stress tolerance or resistance in plants are not known. In this communication, we report *p*-nitrophenyl acid phosphatase (APH) activity from germinating barley seeds under different levels of osmotic stress. Two varieties of barley, C-164 and C-138, which are recognised suitable for irrigated and unirrigated cultivation respectively, were taken for these studies.

Seeds of *Hordeum vulgare* L., var. C-138 and C-164 were obtained from the Department of Plant Breeding of this University. The seeds were germinated in distilled water or solutions of polyethylene-glycol (6000) of 1, 3, and 5 atmosphere osmotic pressures<sup>5</sup> in petriplates under sterilized conditions, in a germinating chamber maintained at 20° C. Enzyme was extracted at 0–4° C from thoroughly washed material, by grinding in 0.05 M Tris (pH 7.4) containing 5 mM 2-mercaptoethanol. The

slurry was centrifuged at  $15,000 \times g$  for 30 min in a cold centrifuge and supernatant was collected for enzyme assay. Proteins were estimated by using Folin-Ciocalteu reagent<sup>6</sup> and crystalline bovine serum albumin as a standard.

Acid phosphatase was assayed using *p*-nitrophenyl phosphate as substrate<sup>7</sup> and activity has been expressed as  $\Delta A_{410} \times \text{min}^{-1} \times \text{mg}^{-1}$  protein.

Barley C-164, which grows well under irrigated conditions, had much higher (30 times) APH activity as compared to the unirrigated variety C-138 (Table I). During germination, APH activity of

TABLE I  
*Water stress and p-nitrophenyl acid phosphatase activity in germinating barley seeds*

| Imbibition<br>(hr)   | Water stress (atm) |       |      |      |
|--|--------------------|-------|------|------|
|  | 0                  | 1     | 3    | 5    |
| (APH activity $\Delta A_{410} \times \text{min}^{-1} \times \text{mg}^{-1}$ protein) |                    |       |      |      |
| C-164 (irrigated)  |                    |       |      |      |
| 0  | 2.17               |       |      |      |
| 24   | 2.58               | 2.50  | 2.26 | 2.30 |
| 48   | 3.93               | 3.42  | 2.89 | 3.32 |
| 72   | 11.24              | 6.69  | 3.21 | 2.27 |
| 96   | 13.80              | 7.62  | 5.62 | 7.03 |
| C-138 (unirrigated)  |                    |       |      |      |
| 0  | 0.07               |       |      |      |
| 24   | 0.21               | 0.14  | 0.12 | 0.11 |
| 48   | 2.37               | 2.41  | 1.78 | 1.05 |
| 72   | 9.86               | 11.46 | 6.46 | 3.83 |
| 96   | 4.44               | 4.14  | 1.70 | 1.34 |

C-164 continued to increase and at 96 hr, the increase was nearly 6 times, while in C-138, APH

activity increased upto 72 hr only and then declined sharply. Increase in activity in unirrigated variety was 55 (5 atm, 72 hr) to 160 (1 atm, 72 hr) folds, during germination.

Acid phosphatase is an important enzyme in the metabolism of phosphate compounds in plants. Though in irrigated variety the initial (0 hr) APH activity is nearly 30 times more than the unirrigated variety, increase in APH activity during germination under stress, in unirrigated variety is remarkable. Various criteria like proline accumulation<sup>8</sup>, increase in soluble proteins<sup>9</sup> and peroxidase activity<sup>10</sup>, have been proposed as indices for stress tolerant plants. We suggest that the APH activity would serve useful criteria, for selecting plants possessing drought resistance. However, this criteria needs extensive investigation on other crop plants.

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#### POLAROGRAPHIC STUDY OF THE ACID HYDROLYSIS OF SOME CARBONYL DERIVATIVES OF RESACETOPHENONE

POLAROGRAPHIC investigation in moderately concentrated acid solutions carried out by the authors on the oximes of substituted acetophenones revealed that the diffusion current decreased with time. This was shown to be due to the acid hydrolysis of the oxime<sup>1</sup>. The authors, therefore, studied in detail the kinetics of the acid hydrolysis of the substituted acetophenone oximes<sup>2</sup>. As an extension of the earlier work, the authors have now taken up the kinetic study of some carbonyl derivatives of acetophenone and its substituted compounds. The results obtained in respect of the acid hydrolysis of the oxime, phenylhydrazine and semi-carbazone of resacetophenone (2, 4-dihydroxy acetophenone) in

moderately concentrated solutions of hydrochloric acid are presented in this communication. The compounds investigated were prepared by the standard methods<sup>3</sup>, and the purity checked by comparing the physical constants with literature data. All the other chemicals used were of Analar grade. Current measurements at different time intervals were made with an LP 55A Photographic Recording instrument. The procedure employed to monitor the reaction is the same as reported earlier<sup>2</sup>. The first order rate constants ( $k$ ) were calculated from the slopes of log  $i$  vs time plots. The plots are quite linear in all the cases. The rate data are presented in Table I.

TABLE I  
Rate constants for hydrolysis of the carbonyl  
derivatives of resacetophenone at 30° C

| [HCl]<br>(M) | $k \times 10^4 (\text{sec}^{-1})$ |                      |                    |
|--------------|-----------------------------------|----------------------|--------------------|
|              | Oxime                             | Phenylhydra-<br>zone | Semi-<br>carbazone |
| 0.01         | ..                                | 1.46                 | ..                 |
| 0.04         | 0.64                              | 2.12                 | 11.9               |
| 0.07         | ..                                | 2.99                 | ..                 |
| 0.10         | 1.02                              | 3.67                 | 28.1               |
| 0.40         | 1.42                              | 2.69                 | 44.8               |
| 0.50         | 1.45                              | ..                   | ..                 |
| 0.70         | 1.35                              | 2.04                 | 41.8               |
| 0.80         | ..                                | ..                   | 74.7               |
| 1.00         | 1.15                              | 1.29                 | 54.8               |
| 2.00         | 0.69                              | 0.94                 | 52.2               |
| 3.00         | 0.63                              | 0.87                 | 48.0               |

An inspection of the data in Table I has shown that the general behaviour of the hydrolysis is the same with all the three substrates. However, the acid concentration corresponding to the rate maximum is not the same. These values are found to be 0.1, 0.5 and 0.8 M for the phenylhydrazine, the oxime and the semi-carbazone respectively. This difference can probably be attributed to the proton accepting ability of the nitrogen of  $>C=N$  in the three substrates. The similarity of behaviour suggests that the same mechanism as reported earlier for the oxime operates in the other two substrates also. But the rate at any acid concentration is in the order semi-carbazone  $>$  phenylhydrazine  $>$  oxime. This cannot evidently be attributed to the steric hindrance at the reaction moiety, since

it is expected to be less for the oxime and more for the phenylhydrazone. The chelation present in the three substrate molecules may probably be responsible for the observed rate order. This implies that the strength of hydrogen bond between the phenolic oxygen and the nitrogen of the  $>C=N$  is in the order semicarbazone  $<$  phenylhydrazone  $<$  oxime. This receives support from the frequency of the phenolic group in the infrared spectra of the three compounds in nujol or KBr.

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### FERROZINE—A REAGENT FOR RUTHENIUM(III) AND OSMIUM(VIII)

STOOKEY's iron reagent ferrozine<sup>1</sup>—the disodium salt of 3-(2-pyridyl)-5, 6-bis(4-phenylsulphonic acid)-1, 2, 4-triazine which the present authors developed<sup>2</sup> for determination of Cu(I) and Co(II)—has now been further used for spectrophotometric determination of Ru(III) and Os(VIII).

Standard solution of ferrozine (0.02 M) was prepared by dissolving 10.280 gm/litre of the compound in double distilled water. Standard Ru(III) and Os(VIII) solutions were prepared from  $RuCl_3 \cdot 3H_2O$  in 1 N HCl and osmic acid in 5 N NaOH and standardized.

Preliminary studies showed that acetate buffered Ru(III) and Os(VIII) solutions formed coloured complexes on being heated with ferrozine. The characteristics of the complexes were investigated (Table I). The two metals were subsequently determined and the effects of diverse ions investigated.

**Procedure for determination of ruthenium.**—To a suitable aliquot of Ru(III) solution, add 7.00 ml ferrozine followed by 5.0 ml of acetate-HCl buffer (pH 2.5). Heat the solution on steam bath for 3 hr, cool it, make up the volume and measure the absorbance at 480 nm against reagent blank. Compare the absorbance with a standard curve to calculate the amount of ruthenium.

**Procedure for determination of osmium.**—To a suitable aliquot of osmium solution, add 8.0 ml of ferrozine and adjust the pH to 2.5 using sodium

acetate and HCl. Heat the solution on steam bath for 4 hr, make up the volume and measure the absorbance at 510 nm against the reagent blank. Calculate the amount of osmium present with the help of a standard curve.

TABLE I  
*Characteristics of metal complexes with ferrozine*

|  | Ru (III)-<br>complex | Os (VIII)-<br>complex |
|--|----------------------|-----------------------|
| Colour   | Brown                | Pink                  |
| $\lambda_{max}$ (nm)                               | 480                  | 510                   |
| $\epsilon_{max}$                                   | 31,500               | 14,400                |
| Ferrozine needed for<br>full colour development    | 120-times            | 200-times             |
| pH range for maximum<br>and constant<br>absorbance | 2.0-3.2              | 2.0-3.4               |
| Beer's law range ( $\mu$ g/ml)                     | up to 2.8            | up to 6.0             |
| Composition<br>(metal: ferrozine)                  | 1:2                  | Not ascertained       |
| Sandell's sensitivity                              | 0.0331               | 0.011                 |
| Standard deviation<br>(with 8 samples)             | 0.0103               | 0.007                 |
| Relative mean deviation<br>(in parts per thousand) | 14.7                 | 6.1                   |
| Coefficient of variation                           | 2.48                 | 1.57                  |

The effects of various ions was examined in the determinations. In case a precipitation occurred, it was centrifuged off and the absorbance of decanted solution was measured after bringing it to the desired volume. In the estimation of 1.4  $\mu$ g/ml of ruthenium, the following ions could be tolerated to the limits given in parentheses (in  $\mu$ g/ml): Halides (except  $I^-$ ),  $NO_3^-$ ,  $SO_4^{2-}$ ,  $Mg^{2+}$ , alkaline earths,  $Zn^{2+}$ ,  $Cd^{2+}$  or  $Hg^{2+}$  (1000);  $Al^{3+}$  or  $Tl^+$  (500);  $PO_4^{3-}$  or  $Ag^+$  (200); tartrate, citrate or  $BO_3^{3-}$  (150);  $Sn^{2+}$ ,  $Pb^{2+}$ ,  $Bi^{3+}$ ,  $Mo^{6+}$  or  $Mn^{2+}$  (100);  $I^-$  (75);  $V^{3+}$  or  $V^{5+}$  (50);  $CNS^-$ ,  $Rh^{3+}$  or  $Pt^{4+}$  (20);  $Cu^{2+}$ ,  $Cr^{3+}$ ,  $UO_2^{2+}$  or  $Pd^{2+}$  (10) and  $Ni^{2+}$  or  $Ir^{3+}$  (5). The presence of  $NO_2^-$ ,  $C_2O_4^{2-}$ ,  $SO_3^{2-}$ ,  $Cu^{2+}$ ,  $Fe^{2+}$ ,  $Fe^{3+}$ ,  $Co^{2+}$ ,  $Cr^{6+}$  or  $Os^{8+}$  caused interference.

In determination of 1.0  $\mu$ g/ml of osmium, the following ions were tolerated to the extents given in parentheses (in  $\mu$ g/ml): Halides,  $NO_3^-$ ,  $SO_4^{2-}$ ,  $PO_4^{3-}$ ,  $Mg^{2+}$ , alkaline earths,  $Zn^{2+}$ ,  $Cd^{2+}$  or  $Hg^{2+}$  (500);  $Ag^+$ ,  $Al^{3+}$  or  $Tl^+$  (100); tartrate, citrate,  $BO_3^{3-}$ ;  $Sn^{2+}$ ,  $Pb^{2+}$ ,  $Bi^{3+}$ ,  $V^{4+}$ ,  $V^{5+}$ ,  $Mo^{6+}$  or  $Mn^{2+}$  (50);

$\text{UO}_2^{2+}$ , or  $\text{Cr}^{3+}$  (10) and  $\text{Ni}^{2+}$  (5). The ions  $\text{NO}_2^-$ ,  $\text{CNS}^-$ ,  $\text{C}_2\text{O}_4^{2-}$ ,  $\text{SO}_3^{2-}$ ,  $\text{Cu}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Rh}^{3+}$ ,  $\text{Ru}^{3+}$ ,  $\text{Pd}^{2+}$ ,  $\text{Ir}^{3+}$  and  $\text{Pt}^{4+}$  caused serious interference.

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### SYNTHESIS OF SOME HETEROCYCLES DERIVED FROM 1-ACYL-4-SUBSTITUTED THIOSEMICARBAZIDES

VARIOUS 1-acyl-4-substituted thiosemicarbazides<sup>1-3</sup> have been found to possess interesting biological properties. It has also been observed that the cyclic products such as oxadiazole, thiadiazole and mercapto-triazole that may be derived from these thiosemicarbazides have also been reported to exhibit

antitubercular<sup>1</sup>, bacteriostatic<sup>2</sup>, hypoglycemic<sup>4,5</sup>, diuretic<sup>6</sup>, antiviral<sup>7</sup> and antifungal<sup>8</sup> action. On this basis a study of these heterocycles derived from 1-acyl-4-substituted thiosemicarbazides was considered to be of interest as they may show pharmacodynamic properties.

The 1-acyl-4-substituted thiosemicarbazides ( $\text{RCONHNH-C-NHR}'$ ) on oxidation with iodine<sup>9</sup>



in potassium iodine cyclise to furnish corresponding 1, 3, 4-oxadiazoles. Cyclodehydration<sup>10</sup> with conc. sulphuric acid gives rise to corresponding 1, 3, 4-thiadiazoles and on refluxing with sodium hydroxide<sup>11</sup> (4%) 5-mercapto-1, 2, 4-*H* triazoles are obtained.

In the present investigations seven thiosemicarbazides, viz., 1-(5'-bromocoumariloyl)-4-(phenyl, 4-chlorophenyl, 4-methoxyphenyl, cyclohexyl, 4-bromophenyl, 2-methylphenyl or 4-methylphenyl) thiosemicarbazides prepared earlier<sup>12</sup> have been cyclised to give corresponding 1, 3, 4-oxadiazoles, 1, 3, 4-thiadiazoles and 5-mercapto-1, 2, 4-*H* triazoles derivatives for the evaluating their biological activities. The details of the prepared heterocycles (I, II and III) are recorded in Tables I, II and III respectively.

The antitubercular and antifungal activities of some of these compounds have been evaluated.

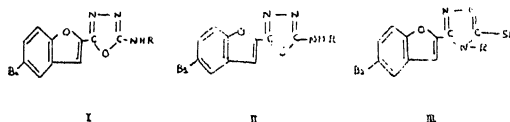


TABLE I

Characteristics of 2-substitutedamino-5-(5'-bromo-2'-benzofuranyl)-1, 3, 4-oxadiazoles, I

| Sl. No. | R               | Yield % | M.P. °C | Mol. formula  | % Nitrogen |          |
|---------|-----------------|---------|---------|---|------------|----------|
|         |                 |         |         |   | Found      | Required |
| 1.      | Phenyl          | 62      | 251 (d) | $\text{C}_{16}\text{H}_{10}\text{N}_3\text{O}_2\text{Br}$ | 11.92      | 11.79    |
| 2.      | 4-Chlorophenyl  | 58      | 210     | $\text{C}_{16}\text{H}_8\text{O}_2\text{N}_3\text{BrCl}$  | 10.62      | 10.75    |
| 3.      | 4-Methoxyphenyl | 60      | 198     | $\text{C}_{17}\text{H}_{12}\text{O}_3\text{N}_3\text{Br}$ | 11.02      | 10.86    |
| 4.      | Cyclohexyl      | 63      | 237     | $\text{C}_{16}\text{H}_{16}\text{O}_2\text{N}_3\text{Br}$ | 11.52      | 11.60    |
| 5.      | 4-Bromophenyl   | 55      | 220     | $\text{C}_{16}\text{H}_8\text{O}_2\text{N}_3\text{Br}_2$  | 9.74       | 9.65     |
| 6.      | 2-Methylphenyl  | 57      | 247 (d) | $\text{C}_{17}\text{H}_{12}\text{O}_2\text{N}_3\text{Br}$ | 11.46      | 11.35    |
| 7.      | 4-Methylphenyl  | 60      | 224     | $\text{C}_{17}\text{H}_{12}\text{O}_2\text{N}_3\text{Br}$ | 11.42      | 11.35    |

d = decomposes.

TABLE II

Characteristics of 2-substitutedamino-5-(5'-bromo-2'-benzofuranyl)-1, 3, 4-thiadiazoles, II

| Sl. No.            | R  | Yield % | M.P. °C | Mol. formula  | % Nitrogen |          |
|--------------------|----|---------|---------|---|------------|----------|
|                    |    |         |         |   | Found      | Required |
| 1. Phenyl          | .. | 60      | 228     | C <sub>16</sub> H <sub>10</sub> N <sub>3</sub> OBrS               | 11.20      | 11.29    |
| 2. 4-Chlorophenyl  | .. | 65      | 247     | C <sub>16</sub> H <sub>9</sub> ON <sub>3</sub> SB <sub>2</sub> Cl | 10.47      | 10.33    |
| 3. 4-Methoxyphenyl | .. | 70      | 251     | C <sub>17</sub> H <sub>12</sub> O <sub>2</sub> N <sub>3</sub> BrS | 10.32      | 10.44    |
| 4. Cyclohexyl      | .. | 68      | 231     | C <sub>16</sub> H <sub>16</sub> ON <sub>3</sub> BrS               | 11.01      | 11.11    |
| 5. 4-Bromophenyl   | .. | 61      | 258     | C <sub>16</sub> H <sub>9</sub> ON <sub>3</sub> Br <sub>2</sub> S  | 9.52       | 9.31     |
| 6. 2-Methylphenyl  | .. | 64      | 244     | C <sub>17</sub> H <sub>12</sub> ON <sub>3</sub> BrS               | 10.76      | 10.88    |
| 7. 4-Methylphenyl  | .. | 66      | 261     | C <sub>17</sub> H <sub>12</sub> ON <sub>3</sub> BrS               | 10.94      | 10.88    |

TABLE III

Characteristics of 3-(5'-bromo-2'-benzofuranyl)-4-substituted-5-mercapto-1, 2, 4-H triazoles, III

| Sl. No.            | R  | Yield % | M.P. °C | Mol. formula  | % Nitrogen |          |
|--------------------|----|---------|---------|---|------------|----------|
|                    |    |         |         |   | Found      | Required |
| 1. Phenyl          | .. | 63      | 226     | C <sub>16</sub> H <sub>10</sub> ON <sub>3</sub> BrS               | 11.43      | 11.29    |
| 2. 4-Chlorophenyl  | .. | 66      | 243     | C <sub>16</sub> H <sub>9</sub> ON <sub>3</sub> BrSCl              | 10.50      | 10.33    |
| 3. 4-Methoxyphenyl | .. | 62      | 212     | C <sub>17</sub> H <sub>12</sub> O <sub>2</sub> N <sub>3</sub> BrS | 10.32      | 10.44    |
| 4. Cyclohexyl      | .. | 60      | 230     | C <sub>16</sub> H <sub>16</sub> ON <sub>3</sub> BrS               | 11.01      | 11.11    |
| 5. 4-Bromophenyl   | .. | 66      | 271 (d) | C <sub>16</sub> H <sub>9</sub> ON <sub>3</sub> Br <sub>2</sub> S  | 9.52       | 9.31     |
| 6. 2-Methylphenyl  | .. | 63      | 255     | C <sub>17</sub> H <sub>12</sub> ON <sub>3</sub> BrS               | 11.02      | 10.88    |
| 7. 4-Methylphenyl  | .. | 65      | 248     | C <sub>17</sub> H <sub>12</sub> ON <sub>3</sub> BrS               | 11.00      | 10.88    |

d = decomposes.

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# POLYPHENOLS OF THE LEAVES OF *OCHNA JAPOTAPITA*

*Ochna japonica* Linn.<sup>1</sup> (Syn. *O. squarrosa* Linn., fam. Ochnaceae) is a small tree whose different parts are useful in the indigenous system of medicine<sup>1,2</sup>. Recently Okigawa *et al.*<sup>3</sup> reported the isolation of a biflavone, ochnaflavone from the acetone extract of the leaves. In continuation of our work on the leaf flavonoids of Indian Medicinal Plants, we have systematically examined the leaves of *O. japonica* and the results are recorded here.

Shade dried leaves of *O. japonica* collected from the Annamalai University Campus, Chidambaram, were extracted with hot acetone followed by rectified spirit, and the concentrates were worked up separately. The alcohol concentrate was divided into three fractions by partition using ether, ethyl acetate and ethyl methyl ketone. The ether fraction showed the presence of minute quantities of apigenin and luteolin. The ethyl acetate and ethyl methyl ketone extracts contained the same components, and hence they were mixed and concentrated to yield a cream coloured solid. This was found to be a mixture of two substances, which were separated into acetone solubles and acetone insolubles. The acetone soluble component was thrice recrystallised from acetone-chloroform to yield a buff coloured powder, blackening above 220°,  $[\alpha]_D^{25} + 32.7^\circ$  (acetone). It had  $\lambda_{max}$  278 nm and  $\nu_{max}^{KBr}$  3380 (broad), 1620, 1540, 1460, 1380, 1300, 1290 and 1110  $cm^{-1}$  characteristic of proanthocyanidins. The NMR spectrum of the compound and the NMR and IR spectra of its acetate further confirmed its proanthocyanidin nature. On treatment with 5 N.HCl in ethanol, it yielded cyanidin (identified by  $\lambda_{max}$  and co-chromatography with an authentic sample). Thus, the original compound was identified as a procyanidin. Fuller characterisation is in progress.

The acetone insoluble fraction was recrystallised from aqueous methanol to yield a light yellow solid, answering all the tests for flavonoids. It was found to be a mixture of three closely related glycoflavones by paper chromatography. They were separated into pure components by preparative PC using *n*-butanol : 27% acetic acid (1 : 1) mixture as developing solvent. Each component was identified by  $\lambda_{max}$ , preparation of its acetate, resistance to hydrolysis with 7% H<sub>2</sub>SO<sub>4</sub>, treatment with HI in phenol, R<sub>f</sub> and co-chromatography with authentic samples. Vitexin (apigenin-8-C-glucoside), orientin (luteolin-8-C-glucoside) and isoorientin (luteolin-6-C-glucoside) were thus isolated and identified, in almost equal proportion.

The acetone extract of the leaves contained larger quantities of the procyanidin and small quantities of

the flavonoids. No biflavone could be detected by us.

This is the first record of occurrence of glycoflavones in the *Ochnaceae*. Our isolation of procyanidin and glycoflavones from the leaves of *O. japonica* collected in South India is in contrast with the isolation of ochnaflavone (a biflavonoid) from the North Indian sample. The formation of procyanidin (flavan condensation) or ochnaflavone (flavone condensation) appears to be a favourable polyphenolic condensation process taking place in *O. japonica*. Very recently, the leaves of *O. squarrosa* have been reported to contain only sitosterol<sup>4</sup>; no flavonoid has been isolated<sup>5</sup>.

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## A NEW PHOTOMETRIC METHOD FOR THE DETERMINATION OF TERVALENT GOLD WITH NICOTINIC AND BENZOIC ACID HYDRAZIDES

BEAMISH<sup>1</sup> AND BOLTZ<sup>2</sup> have critically reviewed the spectrophotometric methods for the determination of tervalent gold. A perusal of these reviews reveals that the reported spectrophotometric methods are based on the formation of coloured products or colloidal gold by reaction with a variety of ligands. The present investigation is based on the formation of a water soluble pink coloured product when tervalent gold is treated with an excess of either nicotinic acid hydrazide (NAH) or benzoic acid hydrazide (BAH) in an alkaline solution. The colour system obeys Beer's law at 520-530 nm in the range 4.0-36.0  $\mu g$  per ml of gold. The new method has advantages in that (1) as low as 4.0  $\mu g$  gold per ml can be determined with a coefficient of variance of 0.185 with NAH and 0.22 with



BAH. (2) The colour formation is quite rapid and is stable over a period of 20 hours.

coloured product obtained with NAH and BAH has the following absorption characteristics :

|  | NAH  | BAH  |
|--|--|--|
| Beer's law limits                        | ... 4.0-36.0 $\mu\text{g/ml}$                            | 4.0-36.0 $\mu\text{g/ml}$                            |
| Effective photometric range <sup>†</sup> | ... 21.0-36.0 $\mu\text{g/ml}$                           | 23.0-36.0 $\mu\text{g/ml}$                           |
| Molar absorptivity                       | ... $3515 \pm 15 \text{ lit. mole}^{-1} \text{ cm}^{-1}$ | $3065 \pm 20 \text{ lit. mole}^{-1} \text{ cm}^{-1}$ |
| Sandell sensitivity <sup>‡</sup>         | ... 0.056 $\mu\text{g/ml}$                               | 0.065 $\mu\text{g/ml}$                               |

**Reagents.**—A 1% gold (III) chloride (Johnson Mathey Co., London), in 1.0 M hydrochloric acid is prepared and standardised iodometrically<sup>2</sup>.

0.05 aqueous solutions of nicotinic and benzoic acid hydrazides are prepared from Fluka's pure samples. These solutions are rendered alkaline just before mixing with the gold (III) solution, such that the net alkalinity of the reagent solution is 0.25 M.

All other reagents are of analytical reagent quality.

**Apparatus.**—A Hilger Spekker absorptiometer, model H-760 with filter No. 4 (maximum transmission 520 nm) and optically matched glass cuvettes is used for quantitative absorption data. Micro-pipettes are used for measuring the fractional volumes of solutions.

**Procedure for detection of gold (III).**—To a mixture of 0.2 ml of the reagent solution and 0.1 ml of 0.1 M sodium hydroxide taken in the cavity of a spot plate, 0.05 ml of gold (III) solution is added and the total volume made upto 0.5 ml with deionised water. A pink colour which develops on mixing the reagents in the cavity of the spot plate confirms the presence of gold. The identification and dilution limits are found to be as follows :

|                        | Using NAH                   | Using BAH                   |
|------------------------|-----------------------------|-----------------------------|
| Identification limit : | 1.0 $\mu\text{g}$ in 0.5 ml | 2.0 $\mu\text{g}$ in 0.5 ml |
| Dilution limit :       | $1 : 5 \times 10^4$         | $1 : 2.5 \times 10^4$       |

**Procedure for the photometric determination of gold (III).**—To an aliquot of the gold (III) solution (containing 4.0-36.0  $\mu\text{g/ml}$ ) taken in a 25 ml volumetric flask, 2.0-6.0 ml of freshly mixed alkaline reagent solution (either NAH or BAH) is added and the mixture is diluted to 25 ml with deionised water. The absorbance of the resulting pink coloured solution is measured against a water blank in an Hilger Spekker absorptiometer using Filter No. 4 and referred to a standard calibration curve.

For accurate results, it was found necessary to follow the above order of mixing the reagents as otherwise, addition of alkali to a mixture of gold (III) and hydrazide results in the formation of colloidal gold, thus leading to erroneous results. A 20-fold excess of the reagent is found necessary for the rapid development of colour. The pink

**Interferences.**—1000-fold excess of  $\text{Al}^{3+}$ , 500-fold excess of  $\text{Cd}^{2+}$ ,  $\text{Cr}^{3+}$ , 200-fold excess of  $\text{Cu}^{2+}$ ,  $\text{VO}^{2+}$ ,  $\text{UO}^{2+}$ , 100-fold excess of  $\text{Ni}^{2+}$ , 50-fold excess of  $\text{Th}^{4+}$ ,  $\text{Ru}^{3+}$ ,  $\text{Cr(VI)}$  and 10-fold excess of  $\text{Fe}^{2+}$ ,  $\text{Ti}^{3+}$ ,  $\text{Pd}^{2+}$ ,  $\text{Pt(IV)}$  Os(VIII) do not interfere.  $\text{Cu}^{2+}$  interferes in all proportions and should be eliminated prior to the determination of gold.

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### ON THE BOEHM LAMELLAE

THE deformational features associated with the quartz grains are the deformation lamellae, called the Boehm lamellae, and the undulatory extinction bands. The author recognised both these features in the quartzites of Satnur, Bangalore District, India. The Boehm lamellae were observed in only a few of the several grains selected for studying the quartz-axes orientation. The grains were tilted on the horizontal axes of the Universal Stage in their various positions to recognise the presence of the lamellae. It is to be noted that, while undulatory bands are present in all the quartz grains, the Boehm lamellae are observed only in a few grains. When mutually associated, the boundary surfaces of the undulatory bands lie close to  $[0001]$  and the Boehm lamellae consistently intersect  $[0001]$  at high angles. The angle between the quartz axes and the lamellae poles, in several grains in Satnur quartzites, was found to have a narrow range of  $28^\circ$  to  $36^\circ$ . Sander (1948 and 1950, p. 362) and Fairbairn (1949, p. 14) give angles varying from  $7^\circ$  to  $36^\circ$ . Fairbairn refers to the irrational crystallographic

orientation of the lamellae being restricted in character, because of its limit between  $7^\circ$  and  $36^\circ$ . In the Satnur quartzites, this irrational orientation is more restricted than in the examples cited by Sander and Fairbairn, and indicates a higher degree of preferred orientation.

The relationship between the Boehm lamellae and the undulatory extinction bands and their origin has been discussed by several workers. Sander (1930) said that the Boehm lamellae are due to translation-gliding on a flat rhombohedral plane. Fairbairn (1939) suggested the horizontal edge  $[m:r]$  as the single possible glide direction in quartz. In the Satnur quartzite, it may be assumed, the quartz grains have glide directions parallel to  $[m:r]$  and have glide planes which make an angle of  $28^\circ - 36^\circ$  with the base (0001). The lamellae are thus subparallel to (0001) and approximately normal to the undulatory extinction bands.

Another aspect of the Boehm lamellae, found in the Satnur quartzites is their slightly curved nature. At each change of direction of the lamellae at the bends, the undulatory bands show a corresponding divergence, such that the value of the angle  $\alpha$  is constantly maintained in a single grain. This feature is similar to the one noted by Mugge as quoted by Fairbairn (1949, p. 129). This establishes a close relationship between the undulatory bands and the Boehm lamellae.

Lamellae in the quartz grains of Satnur quartzites are never found to occur alone, but are found associated with the undulatory extinction bands, while the latter occur alone. This lends support to the view that the lamellae must be a later phenomenon. Riley (1947) and Turner (1963) also opine that the lamellae would be developed at a late stage in the deformation history of the rock.

An analysis of these views, *vis-a-vis* the findings made on the Satnur quartzites would suggest the contemporaneous development of lamellae and band, and that, if only a few of the quartz grains in these rocks were to show lamellae, it must be due to the fact that only those quartz grains with their axes favourably disposed for glide mechanism will be sheared into lamellae.

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## EXUDATION OF FOLIAR APPLIED PHOSPHORUS ( $^{32}\text{P}$ ) THROUGH ROOTS OF COFFEE PLANTS

A KNOWLEDGE of the exudation of foliar applied plant nutrients through roots of plants will not only help to assess the efficiency of the chemical in the plant system but also to understand the nature of interactions between plant roots and soil micro-organisms<sup>1</sup>. Exudation of various chemicals by roots of different plant species has been reported<sup>2,3</sup>. The incorporation of foliar applied  $^{14}\text{C}$ -glucose and  $^{32}\text{P}$ -phosphate into the root exudates of sorghum (*Sorghum vulgare* Pers.) has also been demonstrated<sup>4</sup>. In this communication, the exudation of foliar applied phosphorus ( $^{32}\text{P}$ ) through roots of two coffee species is reported.

Arabica (*Coffea arabica* L. cv. S. 795) and robusta (*C. canephora* Pierre. cv. S. 274) plants raised initially in polythene nursery bags containing a mixture of jungle soil + farmyard manure + sand (6 : 2 : 1) were transferred to closed polythene containers with bent glass tubes attached for aeration and filled with Hoagland and Arnon<sup>5</sup> nutrient solution No. 2 with the recommended doses of micro-nutrients. The plants were grown at  $26^\circ\text{C}$  and 2,000 foot candles light intensity during daytime (for 8 hr) in a glass house. There were six replications in each coffee species. When the plants were eight months old, 0.2 ml aqueous solution (pH 4.5) of radioactive phosphorus ( $^{32}\text{P}$ ), in the form of sodium dihydrogen orthophosphate, containing  $50\text{ }\mu\text{C}$  of  $^{32}\text{P}$  was applied to each plant, at the rate of two drops to each of the top six leaves avoiding bud leaves. The plants were removed from the growth medium 48 hr after the radio-phosphorus treatment. The nutrient solution was then filtered free of any solid debris, and 2 ml of the filtrate was taken in an aluminium planchet, dried under infra-red heat and the radioactivity monitored in an end-window G.M. counter, and the quantity of  $^{32}\text{P}$  exuded through roots was calculated.

The results in Table I indicate that more radioactive phosphorus was exuded by the roots of *canephora* plants than *arabica*, during the period of 48 hr after foliar feeding. The phosphorus metabolism of lateral roots was found to be greater in

*canephora* than in *arabica*<sup>6,7</sup>. The root system of *canephora* is more shallow and fibrous, tufty and grows extensively with greater network of feeder roots than the root system of *arabica*<sup>7</sup>. These factors might have contributed to greater exudation of phosphorus through *canephora* roots. It should be interesting to study the relationship between the exudation of P in *canephora* and its tolerant nature to many soil-borne fungal pathogens and parasitic nematodes.

TABLE I

Exudation of foliar applied phosphate (<sup>32</sup>P) through roots of coffee plants

| Coffee species | <sup>32</sup> P activity exuded (cpm) | % of applied activity exuded | Quantity of <sup>32</sup> P exuded (μg of P) |
|----------------|---------------------------------------|------------------------------|--|
| Arabica        | 1366±115                              | 0.035                        | 0.016  |
| Robusta        | 8033±276                              | 0.204                        | 0.094  |

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# OBSERVATION ON THE MORTALITY OF THE MONARCH BUTTERFLY *DANAUS CHRYSIPPUS* (LINNAEUS) INFECTED BY *STURMIA CONVERGENS* (WIEDEMANN) (DIPTERA: TACHINIDAE)

ENTOMOPHAGOUS parasitic tachinids select the larvae and adults of Coleoptera, Orthoptera and Hemiptera as their host, but most often parasitise Lepidopteran larvae<sup>1</sup>. Ramakrishna Ayyar<sup>1</sup>, who reported several tachinids on the Lepidopteran larvae, has not mentioned the occurrence of the tachinid *Sturmia convergens* on any danid larvae. This note deals with the occurrence of *S. convergens* on the monarch butterfly *Danaus chrysippus*.

Eggs of *S. convergens* are very small and dark when oviposited on the leaves of *Calotropis gigantea*; these are swallowed along with leaf tissues by *D. chrysippus* larvae. The eggs hatch and the whole larval development occurs within the body of the caterpillar. When the host caterpillar pupates, the fully grown parasitic larva bores its way out, killing the host in the process.

Only one larva comes out from the host. The larva actively wanders for some time (20 minutes to 2 hours) before pupating in the soil or in plain glass terrarium.

Adult *S. convergens* lives for  $3 \pm 1.5$  days without feeding. The morphological features of the adult agree in general with those described for *S. convergens* by Clausen<sup>2</sup>.

*D. chrysippus* mainly feeds on the milkweed *C. gigantea*<sup>3</sup>. Latex of the milkweed contains cardiac glycoside poisons such as calotropin, calotoxin and calactin<sup>4</sup>. The danid larvae retain these glycoside poisons at the adult stage to protect themselves from predatory birds. A danid butterfly, that has eaten *C. procera* contains sufficient poison to cause vomiting and accompanying unpleasant experience in 5 predatory birds, the blue jay, *Cyanocitta cristata*; hence the predators, which control the danid population, are limited.

*S. convergens* appears to be one of the most important parasitic agents causing mortality to *D. chrysippus*. Percentage of infection and consequent mortality of laboratory reared *D. chrysippus* were high during the pupal stage. During 1973 and 1974, *D. chrysippus* populations suffered 15.4 and 12.6% mortality. In the initial 4 instars, the mortality was nil and less than 0.5% in the final instar (Table I).

The presence of *S. convergens* larva becomes apparent mostly in the pupal stage of the host; this may be due to the following reasons: More than 80% of the total food required is consumed by *D. chrysippus* larvae during the final two instars<sup>5,6</sup>; hence the chances of the egg of *S. convergens* being consumed by the host are more

TABLE I

*Incidence of Sturmia convergens in the monarch butterfly Danaus chrysippus larvae*

| Life stage    | Incidence (%) |      |
|---------------|---------------|------|
|               | 1973          | 1974 |
| First instar  | 0.0           | 0.0  |
| Second instar | 0.0           | 0.0  |
| Third instar  | 0.0           | 0.0  |
| Fourth instar | 0.0           | 0.0  |
| Fifth instar  | 0.3           | 0.4  |
| Pupa          | 15.4          | 12.6 |

during these 2 instars. Even if *S. convergens* eggs gain entry into the host of first or second instar stage, the hatched parasitic larvae may not find sufficient food, as *D. chrysippus* larvae rapidly grow during the final 2 instars only.

The tachinid flies have been shown to be one of the most important agents in regulating the number of Lepidopteran larvae; for instance, nearly 70% of the corn borer *Zeadiatraea grandiolella* was parasitized by the tachinid *Lydella stabulans grisescens*<sup>7</sup>. The highest incidence of *S. convergens* was 15% on *D. chrysippus*. Since *D. chrysippus* is hardly controlled by any other biological agents, *S. convergens* is important for the effective control measure of the danid larvae.

The grasshopper *Poecillocerus pictus*, which exclusively feeds on *C. gigantea*, is also known to accumulate glycoside poisons<sup>4</sup>, and therefore the predators controlling *P. pictus* are very limited. *P. pictus* is also known to be parasitized by the sarcophagid fly *Blaesoxipha kaestneri*. About 11% of the grasshopper population is reported to get killed by *B. kaestneri*<sup>8</sup>. Surprisingly both *P. pictus* and *D. chrysippus*, who have very limited predators, are subjected to a mortality of only 11 and 15% by the respective parasitic flies. It is very likely that there may be other dipterans which parasitize and control *P. pictus* and *D. chrysippus*.

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#### INDUCED CHANGES IN OVIPOSITION BY JUVENILE HORMONE ANALOGUE IN THE MOSQUITO, *ANOPHELES STEPHENSI*

HORMONAL influence in the reproduction of mosquitoes has been studied by several workers<sup>1-4</sup>. However, sterilization through juvenile hormone mimics has been reported only in *Aedes aegypti*<sup>5</sup>. In the course of our investigations on the effect of juvenilizing substances on insects, certain abnormalities in the eggs oviposited by the female mosquitoes, *Anopheles stephensi*, were observed following the treatment with the juvenile hormone analogue (JHa). Isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate.

The experimental mosquitoes, *Anopheles stephensi*, were taken from the cultures maintained in the laboratory under controlled conditions of temperature and humidity. 500 pupae were collected and allowed to emerge into adults in a cage. Freshly emerged adults were fed for two days on 10% glucose solution mixed with the JHa. Then the mixture of JHa and glucose was removed and the mosquitoes were fed on the blood of guinea pigs. Thereafter, glucose alone was offered every day and blood meal was given on every alternate day. The experiments were repeated thrice and various concentrations of the JHa in glucose solution (1.0-1%) were tried.

There was some mortality in 1% concentration while it was negligible in 0.1% concentration. After the first blood meal, the mosquitoes oviposited the first batch of eggs on the third day which contained a large number (above 80%) of abnormal eggs. These abnormal eggs were found to be white in colour, compared to the dark normal eggs. The white eggs were smaller in size and were sometimes

devoid of floats or were partially developed in some cases. They usually burst open and the yolk oozed out when even a slight pressure was exerted in the water on which they were laid. Further, these eggs did not hatch into larval forms. The least concentration tested was also effective in producing these abnormal eggs.

When Patterson<sup>9</sup> treated *Aedes aegypti* topically after the blood meal, the mosquitoes laid eggs devoid of chorion, but the effect was temporary. In the present investigation, the JHa was administered orally prior to the first blood meal and the effect was found for a longer period (i.e., in the subsequent batches of eggs as well). Following the treatment with the JHa, the mosquitoes oviposited the abnormal eggs daily for ten days. However, the percentage of the white eggs gradually declined and the eggs were almost normal after the 10th day. Sometimes, certain intermediary eggs with light brown colouration were also observed around the 6th day.

These results indicate, that the JH analogues taken by the female mosquitoes act on the follicle cells of the ovary, thus interfering with the normal formation of the chorion and the development of the oocyte. The effect seems to be more if the JHa is administered prior to the first blood meal in the anautogenous mosquitoes. Further studies on the action of juvenile hormone analogues and their prospective use in insect control are under investigation in this laboratory.

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# TEMPERATURE AS A FACTOR AFFECTING EGG PRODUCTION, OVIPOSITION PERIODS AND ADULT LONGEVITY IN *TROGODERMA GRANARIUM* EVERTS (KHAPRA BEETLE) (COLEOPTERA—DERMESTIDAE)

THERE are some reports on the effect of temperature on the biology of *Trogoderma granarium* Everts (Khapra-beetle). Lindgren *et al.*<sup>1,2</sup> and Sharifi<sup>3</sup> studied the biology of *Trogoderma granarium*. Hadaway<sup>4</sup>, Girish<sup>5</sup> and Uniyal *et al.*<sup>6</sup> made certain contributions towards the effect of temperature on the biology of Khapra beetle. Karnavar<sup>7</sup> studied the mating behaviour and fecundity in *T. granarium*. However, the complete information with regard the effect of temperature on oviposition periods, egg laying, survival of eggs and longevity of the adult is lacking. This has been taken up for detailed study, in the present investigation.

A culture of *T. granarium* was maintained at  $35 \pm 2^\circ \text{C}$  and 70–75% relative humidity on whole wheat grains. Newly emerged beetles of both the sexes were kept in pairs on 4 gm of wheat grain. In the first experiment, set to determine the oviposition periods and egg laying, 15 replicas were run while in the second experiment which was set to determine the incubation period and survival of eggs 10 replicas were run.

A temperature of  $32^\circ \text{C}$  is taken as the optimum for the development of this pest, as the egg laying, survival of eggs and the number of larvae developed from the eggs are maximum at this temperature (Table I).

The results (Table I) reveal that with the increase in temperature from optimum, i.e.,  $32^\circ \text{C}$  to  $40^\circ \text{C}$ , there is a general decrease in pre-oviposition and oviposition periods, longevity of adult mature males and fertilized females, egg laying and survival of eggs. On decreasing the temperature to  $28^\circ \text{C}$ , there is a general increase in pre-oviposition, oviposition and post-oviposition periods and adult duration of mature males and fertilized females. The egg laying and the survival of eggs decreases at the temperature below the optimum.

With the decrease in the temperature, the period of gonad maturation increases which obviously would result in the increase in pre-oviposition and oviposition periods and *vice versa* as also observed by Mathlein<sup>8</sup> and Howe *et al.*<sup>9</sup>, who reported increased periods of oviposition in *Sitophilus granaria* and *Ptinus tectus* respectively, at the temperature below optimum. With the increase in pre-oviposition period the egg laying and adult longevity will also get affected.

The interference in the copulation by temperature may be another reason for low rate of egg laying. This is in accordance to Zwolfer<sup>10</sup> who made similar

TABLE I  
Variations in oviposition periods, egg laying, incubation period and survival of eggs

| Temp. °C | No. of females | Variation in pre-oviposition period (%) | Variation in oviposition period (%) | Variation in post-oviposition period (%) | Variation in adult life of male (%) | Variation in adult life of fertilised females (%) | Decrease in total egg laying (%) |
|----------|----------------|---|-------------------------------------|--|-------------------------------------|---|----------------------------------|
| 28 ±1    | 13             | +112                                    | + 8                                 | +114                                     | ÷67                                 | ÷49   | 40                               |
| 30 ±2    | 14             | + 38                                    | + 9                                 | + 28                                     | ÷35                                 | ÷14   | 11                               |
| 32 ±1    | 13             | Nil                                     | Nil                                 | Nil                                      | Nil                                 | Nil   | Nil                              |
| 35 ±2    | 14             | - 7                                     | -10                                 | + 5                                      | ÷ 6                                 | ÷ 4   | 8                                |
| 37.5±2   | 14             | - 59                                    | -30                                 | + 5                                      | -21                                 | -28   | 36                               |
| 40 ±1    | 14             | - 67                                    | -52                                 | - 1                                      | -40                                 | -37   | 30                               |

TABLE I—(Contd.)

| Temp. °C | No. of females | Mean eggs female ± S.E. | Range of egg laying | Range of incubation period (days) | Variation in mean incubation period (%) | Decrease in survival of eggs (%) | Range of survival of eggs |
|----------|----------------|-------------------------|---------------------|-----------------------------------|---|----------------------------------|---------------------------|
| 28 ±1    | 13             | 31±10                   | 9-47                | 6-8                               | ÷47                                     | 36                               | 20-80                     |
| 30 ±2    | 14             | 47±14                   | 14-74               | 5-7                               | ÷12                                     | 26                               | 30-100                    |
| 32 ±1    | 13             | 53±24                   | 17-109              | 5-6                               | Nil                                     | Nil                              | 50-100                    |
| 35 ±2    | 14             | 48±15                   | 23-71               | 4-6                               | - 9                                     | 12                               | 50-100                    |
| 37.5±2   | 14             | 34±12                   | 19-58               | 3-5                               | -24                                     | 31                               | 40-80                     |
| 40 ±1    | 14             | 37± 9                   | 17-70               | 3-5                               | -28                                     | 36                               | 30-70                     |

+ denotes increase with reference to optimum temperature.

- denotes decrease with reference to optimum temperature.

reports on *Panolis flammea*. Further the reduced egg hatching may be another interference in the copulation by temperature as more unfertilized eggs are likely to be laid at a temperature other than optimum.

Another reason for prolonged adult longevity at a temperature below the optimum could be the low rate of egg laying accompanied by the oosorption as also reported by Phipps<sup>11</sup> and Engelmann<sup>12</sup>.

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### THE GENUS *QUADRUSPINOSPORA* (PROTOZOA:SPOROZOA)—A NEW DEFINITION

IN 1969, Sarkar and Chakravarty<sup>1</sup> created a new genus *Quadruspinospora* to accommodate the cephaline gregarines inhabiting the various parts of the midgut of the grasshopper, *Aelopus* sp. The genus was characterised by "(a) solitary, elongated trophozoite having a subspherical epimerite with eight to 12 stumpy digitiform processes, hemispherical protomerite and granular deutomerite which is broadest immediately behind the septum; (b) spherical nucleus with several karyosomes; (c) spherical, thick-walled gametocysts dehiscing by simple rupture; (d) oval spores with a pair of very long spines at each pole, and (e) intracellular development".

While studying the cephaline gregarines from various insects of this locality<sup>2</sup>, we have observed that grasshoppers of different species are infested with a cephaline gregarine belonging to the genus *Quadruspinospora* Sarkar and Chakravarty. We have already described *Q. chakravartyei*<sup>3</sup> and *Q. atractomorphii*<sup>4</sup> from the grasshoppers *Spathosternum* sp. and *Atractomorpha crenulata* (Fabr.) respectively. Preliminary studies have revealed that three other species of cephaline gregarines of the same genus parasiting the grasshoppers are also likely to be new to science (unpublished data). In all the five species, the number of digitiform processes attached to the epimerite does not conform to the number as given by Sarkar and Chakravarty: in *Q. chakravartyei* the number is 20 to 24, in *Q. atractomorphii* it is 12 to 18, and in the other three species the number varies between 10 to 23. Moreover, the shape of the nucleus in the adult trophozoite is not always spherical but may be subspherical or elliptical also. In all the five species, however, the spore contains a pair of very long spines at each pole which, according to us, is the most diagnostic character of the genus. The intracellular development takes place inside the epithelial cells of the hepatic caeca only. We, therefore, propose a new definition of the genus *Quadruspinospora* to accommodate these parasites under this genus as follows:

(1) Solitary, elongated trophozoite with a subspherical epimerite having a variable number of stumpy digitiform processes; (2) hemispherical protomerite and granular deutomerite broadest immediately behind the septum; (3) spherical, subspherical or elliptical nucleus in the adult trophozoite; (4) thick-walled, spherical gametocysts dehiscing by simple rupture; (5) oval spores with four very long spines, two at each pole; and (6) intracellular development, confined to the epithelial cells of the hepatic caeca.

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### EFFECT OF CERTAIN GRANULAR INSECTICIDES ON THE NODULATION BY NITROGEN-FIXING BACTERIA IN COWPEA (*VIGNA SINENSIS* L.)

It has been reported that application of granular insecticides to soil for the control of insect pests of crops, are known to interfere with the soil flora, particularly microbes including nitrogen fixing bacteria<sup>1,2</sup>. Hence the present study was designed to elucidate the effect of different systemic and non-systemic insecticides on the nodulation in cowpea and the results are presented in this paper.

The experiment was conducted with four replications in the pots with various granular insecticides as described earlier by Swamiappan *et al.* (1974). The seeds were coated thoroughly with the suspension of bacterial culture and seeded in the pots. The plants at 35 and 55 days age were carefully uprooted and the observations were made on the total number and weight of nodules.

The results (Table I) indicate that phorate, an organophosphorus systemic insecticide has enhanced the nodulation by 313% over untreated check. However the weight of nodules has not progressively increased with the increase in the number of nodules. On the other hand, the positive influence in the process of nodulation by the other two granular insecticides, namely, endrin and chlorfenvinphos indicates that the soil application of granular insecticides did not affect either the nodulation or activity of the bacteria. Similar results were reported earlier with the other legumes where it has been found that application of soil insecticides has beneficial effects on the soil flora<sup>1,3,4</sup>. This might possibly be due to the breakdown and degradation of organic phosphorus of the applied toxicants and utilization of the same by the microbes for their metabolic activities.

TABLE I

Influence of certain granular insecticides on the  
nodule formation in cowpea

| Granular Toxicants at 1 lb<br>ai./acre | Number and weight of nodules<br>per plant |                   |
|--|---|-------------------|
|  | Number                                    | Weight in mg      |
| Phorate                                | 120<br>(+ 313)                            | 910.0<br>(+120)   |
| Endrin                                 | 64<br>(+121)                              | 674.0<br>(+63)    |
| Chlorfenvinphos                        | 57<br>(+97)                               | 649.0<br>(+57)    |
| Disulfoton                             | 50<br>(+72)                               | 442.0<br>(+7)     |
| Carbaryl+Lindane<br>(Sevidol)          | 28<br>(-3)                                | 450.0<br>(+9)     |
| Untreated check                        | 29  | 413.0             |
| C.D.                                   | 28<br>(P=0.05)                            | 179.0<br>(P=0.01) |

The figures in parenthesis are per cent decrease or increase over untreated check.

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#### EMBRYO DEVELOPMENT IN *CLEOME* *TENELLA* LINN.

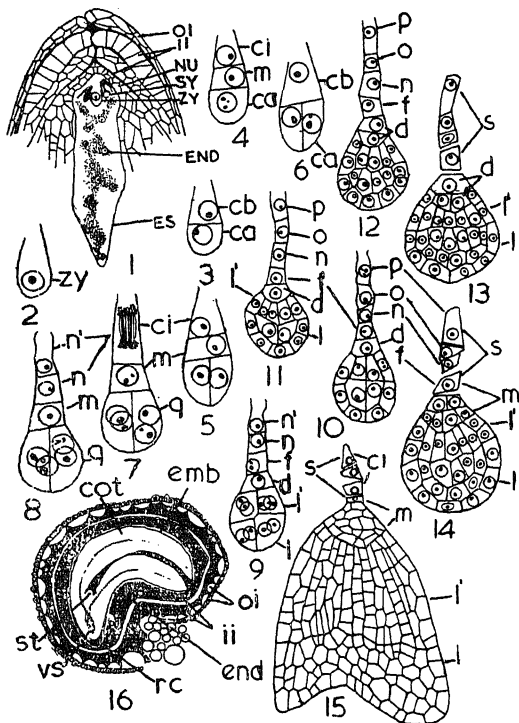
FROM a perusal of the available embryological studies<sup>2-7</sup> it is seen that the Onagrad, Solanad and Caryophyllad types of embryo development are seen among capparidaceous taxa so far investigated. With

reference to *Cleome* this aspect is restricted to a brief description by Mauritzen<sup>2</sup> in *Cleome serrata* and *C. monophylla*, Tiwary<sup>7</sup> on *Cleome viscosa* and a comprehensive account by Raghavan<sup>3</sup> on *Cleome chelidonii*. In this paper a detailed account of the development of the embryo in *Cleome tenella* Linn. is presented and compared with the results recorded by earlier workers on this genus.

The first division of the zygote, which is transverse, takes place only after the formation of a few free endosperm nuclei, resulting in a basal cell *cb* and a terminal cell *ca* (Figs. 1-3). The former (*cb*) divides transversely engendering two superposed cell *m* and *ci*, while the latter (*ca*) divides vertically forming two juxtaposed cells (Figs. 4, 5). Thus an inverted T-shaped 4-celled proembryo (Fig. 5) results at the end of second cell generation. In about 12% of the ovules the division of *ca* precedes *cb* (Fig. 6). Each of the terminal cells divides by a vertical wall at right angles to the first wall (Figs. 7, 8) giving rise to the quadrant stage. At this stage there is a slight elongation of *ci* followed by transverse division resulting in two superposed cells *n* and *n'* (Figs. 7, 8). Meanwhile the quadrants divide transversely to initiate octants disposed in two tiers *l* and *l'* (Fig. 9). Concomitantly, the middle cell *m* of the 4-celled proembryo becomes segmented producing the cells *d* and *f* (Fig. 9). Periclinal divisions in the cells of the octants separate the dermatogen (*de*) from an inner group of cells (Fig. 10). Further periclinal divisions in the cells of *l'* lying inside the dermatogen differentiate an outer periblem (*pe*) and an inner plerome (*pl*) (Figs. 11-14). Concurrently, anticlinal divisions take place in the dermatogen (Figs. 11-14). By the time the periclinal divisions are initiated in *l* and *l'*, the cell *n'* divides to form two superposed cells *o* and *p*, which along with *f* and *n* form a 4-celled suspensor, which subsequently disorganises as the embryo matures (Figs. 10-16). In the tier *l* destined to form the cotyledons and shoot apex, both transverse and vertical divisions are laid down and the embryo eventually passes through the globular and heart-shaped stages (Figs. 13-15). The cell *d* functions as the hypophysis. It divides transversely to procreate two superposed cells which by further divisions produce two groups of cells *iec* and *ico* (Figs. 13, 14). Subsequent divisions in the tier *l* engenders the cotyledonary region (*pco*) and the stem apex (*pvt*). The tier *l'* contributes to the hypocotyledonary region (*phy*) and the initials of the central cylinder of the stem (*icc*) and the tier *m* and its derivatives to the initials of the central cylinder of the root (*iec*) as also the initials of root cap (*ico*). The cells *f*, *n*, *o* and *p* constitute the 4-celled suspensor (Figs. 10-15).



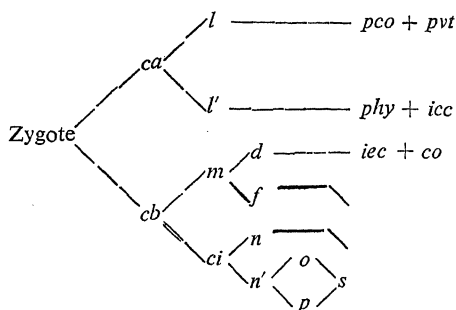
The mature embryo, which extends from the micropylar region to the chalazal end of the mature seed, is curved, dicotyledonous with discernible shoot apex, root, its cap and the vascular supply to the cotyledons (Fig. 16).



FIGS. 1-16. Fig. 1. L.s. micropylar region of ovule showing integuments, nucellus and embryo sac,  $\times 200$ ; note zygote, degenerating synergids and endosperm nuclei. Figs. 2-15. Stages in development of embryo,  $\times 300$ ; Fig. 16. L.s. seed showing mature embryo, endosperm and seed coat,  $\times 30$ . (cot, cotyledons; emb, embryo; end/END, endosperm; ES, embryo sac; ii, inner integument; NU, nucellus; oi, outer integument; rc, root cap; st, stem tip; SY, synergids; VS, vascular supply; ZY, zygote).

Based on the fragmentary account of the embryo development given by Mauritzon<sup>3</sup> for *Cleome serrata* and *Cleome monophylla*, Johansen<sup>2</sup> (p. 142) states 'these species appear to follow the Alyssum variation of Onagrad Type, very closely'. Tiwary<sup>7</sup> records a 3 or 4-celled suspensor for *Cleome viscosa*. The present study on *Cleome tenella* is in conformity with the account given by Raghavan<sup>5</sup> for *Cleome chelidonii* except that the suspensor is 4-celled here, whereas it is 7-celled in *Cleome chelidonii*, and it conforms to the *Lythrum* variation of the Onagrad type of Johansen<sup>2</sup> or conforms to the Megarchetype IV, series A of the First Period of Souéges (*Vide* Crété)<sup>1</sup>. The embryogeny in

*Cleome tenella* Linn. can be recapitulated by the following embryonic formula :



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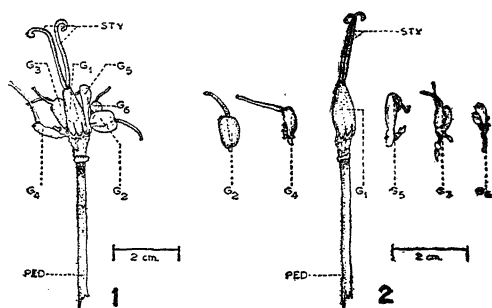
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#### AN ABNORMAL FLOWER OF *DIANTHUS CARYOPHYLLUS* LINN.

ABNORMALITIES in vegetative and floral parts of angiosperms are not of uncommon occurrence. Teratological features of other groups of the plant kingdom have been discussed by earlier workers (Masters, 1869; Goebel, 1897; Candolle, 1905; Costerus and Smith, 1909; Worsdell, 1915, 1916). However, abnormal morphological features in the members of the Caryophyllaceae have not been dealt with in detail, notwithstanding the description of over two dozen cultivated species of *Dianthus* by Bailey (1949) and an exhaustive monograph by Williams (1893). We have observed an abnormality of flower in plants of *Dianthus caryophyllus* L. (Carnation), collected from the Botanical Garden of Meerut College.

The flower is larger than normal and the calyx is fused and form a conical tube (removed in the photograph). The petals are replaced by a whorl of five pistils, surrounding a large, terminal, normal Caryophyllaceous pistil. The terminal as well as the adjacent, laterally placed ovaries show the following structural features :

**Terminal Gynoecium— $G_1$ .**—It is bicarpellary and syncarpous ; the ovary is superior and unilocular with free central placentation. The ovary is whitish at the base and yellow above. Both the styles are long, each with a terminal, curved and hairy stigma (Fig. 2). Seven polyandrous stamens, with unequal filaments, have been observed associated with this gynoecium.



FIGS. 1-2. *Dianthus caryophyllus* L. Fig. 1. Abnormal flower before dissection. Fig. 2. The same flower after dissection showing five laterally placed pistils and a single terminal pistil. ( $G_1$  = Terminal pistil ;  $G_2$ - $G_6$  = Laterally placed pistils ; PED = Pedicel ; STY = Styles.)

**Individual Lateral Gynoecia.**—For the sake of convenience, we have numbered the lateral gynoecia as  $G_2$ ,  $G_3$ ,  $G_4$ ,  $G_5$  and  $G_6$  (Figs. 1-2). The following structural features have been observed in these laterally placed gynoecia :

**Lateral Gynoecium- $G_2$ .**—The ovary is narrow at the base but becomes broad and flat above. A single style arises from a depression and bears a slightly curved stigma. A single stamen with a long thin filament is attached on the innerside of the ovary.

**Lateral Gynoecium- $G_3$ .**—The ovary is narrow at the base and broad in the middle, and tapers towards the top. Two styles arise from a depression at the apex of the ovary. One of the styles is straight, and the other is curved. The curved style has a pointed stigma while the straight one is capitate. Four stamens are associated with this ovary, of which, three are short and the fourth is long.

**Lateral Gynoecium- $G_4$ .**—The ovary resembles that described for lateral gynoecium- $G_3$ . It bears a single style, curving at an angle of about  $90^\circ$  and bearing

a capitate stigma. One well-developed stamen is associated with the gynoecium.

**Lateral Gynoecium- $G_5$ .**—The ovary is broad in the middle and tapers towards both the ends. Two hairy styles with two capitate stigmas are present. Three stamens, one long and two short, are associated with this gynoecium.

**Lateral Gynoecium- $G_6$ .**—The ovary is the smallest of the group. It is broad in the middle, narrowing slightly at both the ends, with two, highly reduced stamens.

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# AN IMPROVED METHOD OF ASSESSING THE EFFICIENCY OF INSECTICIDES AGAINST RICE LEAF ROLLER *CNAPHALOCROCIS MEDINALIS* G.

THE rice leaf roller, hitherto considered as a minor pest has assumed serious pest form with the advent of high yielding rice varieties like I.R. 8 and many workers have attempted previously to control this pest by chemical means<sup>1-6</sup>. The conventional method of assessing the efficacy of insecticides, based only on the damage symptom caused by leaf roller cannot be employed to ascertain the immediate knock down effect of the chemical. Further the effect could be seen only when there is a steady increase in the general infestation level of the pest. Hence an attempt was made to improve this method, taking the population also into account. The present study was conducted at University Farm, Madurai, from October to February and March, (at which time the leaf roller incidence was high) in a simple randomised block design with three replications. The size of the plot was 5 m  $\times$  4 m and the I.R. 8 seedlings were planted with a space of 20  $\times$  15 cm.

The treatments consisted sprays of endrin 0.04%, parathion 0.05%, Heliothox (Toxaphene + DDT) (4 ml/litre), fenthion 0.125%, Trichlorfon 0.12% and Fenitrothion 0.1%. In each plot ten clumps were selected at random at the rate of five in each of the diagonal lines in each plot. The infestation was assessed by counting the total number of leaves and number of leaves showing leaf roller attack (70 days after planting) before spraying. Two days after spraying, the number of the dead caterpillars lying in between three rows of plants were counted in each plot. The rows were selected in such a way that they passed through the maximum infested area. For easy counting, the water level in the field was kept to a minimum. The mean number of dead caterpillars in treated plot was corrected by using the formula :

$$\text{Corrected mortality (C.M.)} = \frac{M_t - M_c}{I_c} \times I_t$$

M — Mean mortality (Number of caterpillars)  
I — Mean infestation (%)  
c — in control plot, t — in treated plot.

The efficacy of the chemical was assessed by using the formula :

$$\text{Efficiency Index (E.I.)} = \frac{\text{C.M.}}{I_t} \times 100.$$

TABLE I  
Efficacy of insecticides against leaf roller  
(Mean of 3 observations)

| Treatments                      | Mean infestation (%) | Mean number of dead caterpillars |           | Efficiency index E.I. |
|---------------------------------|----------------------|----------------------------------|-----------|-----------------------|
|                                 |                      | Actual                           | Corrected |                       |
| Endrin E.C.                     | 51.1                 | 11.33                            | 7.09      | 13.87                 |
| Parathion (Folidol E.C.)        | 47.8                 | 6.67                             | 2.70      | 5.65                  |
| Heliothox E.C., (Toxaphene+DDT) | 67.5                 | 38.00                            | 32.40     | 48.00                 |
| Fenthion (Labaycid E.C.)        | 51.6                 | 6.33                             | 2.05      | 3.97                  |
| Trichlorfon (Dipterex W.S.P.)   | 31.0                 | 9.67                             | 7.10      | 22.90                 |
| Fenitrothion (Folithion E.C.)   | 54.0                 | 10.00                            | 5.52      | 10.22                 |
| Control                         | 40.1                 | 3.33                             | ..        | ..                    |

As the number of dead caterpillars vary with the chemicals as well as the degree of infestation, a correction was used based on the percentage of infestation. As uniform plant population was maintained with uniform spacing, the variation in number of leaves was not much and hence the percentage

of infestation has been used in the formula. Heliothox could cause high rate of kill of leaf roller caterpillars followed by trichlorfon. Endrin and fenitrothion were next best. Parathion was not effective as reported by Gargav *et al.*<sup>2</sup> and Khaire and Bhapkar<sup>3</sup>.

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#### A NEW SPECIES OF *PODOXYPHIUM* (SPHAEROPSIDALES)

AN interesting species of *Podoxyphium* Sp. was collected on rotted fruits of *Sapodilla* at Poona. This proved to be distinct from other known species<sup>1,2</sup>. Hence, the same is described here as new to science.

*Podoxyphium poonensis* sp. nov. Subram. and Rao  
(Fig. 1)

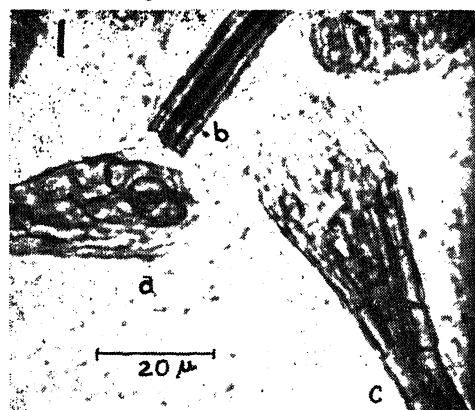


FIG. 1. Morphology of *Podoxyphium poonensis*. (a) Basal; (b) Median parts of the stipe and (c) Pycnidium with scattered spore-mass around.

Mycelium effusae, septatis, ramosis et anastomosis, 3–3.5  $\mu$ . lata, levibus, 9.5–15.2  $\mu$  intervallum inter septa. Stipe singula vel fasciculata ex hypharum erecta, simplicia, recta vel flexuosa, brunneae vel atro-brunneae, 7.6–11.4  $\mu$  lata, ad basi

11–15  $\mu$  inflata. Pycnidia superficialia recta, pallide-brunneis, stipidata, stipe magnit 174.8–228  $\mu$  forma, apiceum versus hyalina; Pycnidiosporae sessilis; continuae, hyalinae, ovoideae vel globosa, unicellularis, 1.5–2  $\mu \times 1 \mu$  [Herb. No. at M.A.C.S. AMH 2421 (Holotype), I.M.I. 186045].

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### A METHOD OF GETTING BACTERIA FREE CULTURE OF BLUE-GREEN ALGA *OSCILLATORIA*

DIFFICULTIES associated in obtaining bacteria free cultures of blue-green algae for critical physiological investigations are ably reviewed by Venkataraman<sup>1</sup>. Bunt<sup>2</sup> used aureomycin. Rubenchick *et al.*<sup>3</sup> also used several methods to get pure cultures of hormone forming blue-green algae.

Great difficulty was faced to get rid slimy *Oscillatoria* of bacteria which take shelter in mucilaginous sheath of the alga. The use of antibiotics, irradiation with UV along have been examined without success. We therefore, thought to remove the gelatinous sheath of the alga with some detergent of biological origin as it would not harm the cells of alga and at the same time loosen or remove the sheath surrounding trichomes of the alga.

40% solution of the soap nuts (fruits of *Sapindus laurifolius*) was prepared by boiling rinds of soap nuts in distilled water, squeezed and filtered. From this stock solution three dilutions were then prepared in the culture medium tubes (1:10, 1:100, 1:10000 V/V), sterilized and inoculated by small portion of the algal growth. Shaking the tubes with glass beads continuously for 30 minutes washing the algal mass three times successively in sterile medium under the aseptic conditions further loosen the sheath. A small portion of the inoculum from each tube was then irradiated under UV light of 160 nm for 10 minutes.

At the end of the irradiation period, a small aliquot was transferred into the sterile culture tube and also streaked on agar plate. Controls of untreated as well as only irradiated (without treatment with soap nuts) were simultaneously kept. The purity of culture was tested by transferring small portion of algal growth into the sterile nutrient

broth and incubated at 30° C. The absence of bacterial turbidity was taken as the criterion for purity of culture.

No turbidity was observed in the cultures treated with the soap nut dilutions of 1:10 and 1:100 with irradiation. But the treatment with soap nut solutions alone showed bacterial turbidity in these two dilutions also.

In a separate experiment the bacteria isolated from the algal association were treated in the concentration (1:10) of soap nut solutions for  $\frac{1}{2}$  hour. After the treatment the bacterial mass was centrifuged and was transferred in tubes of fresh sterile nutrient broth and incubated at 30° C.

No bacterial turbidity was observed in the tubes treated with 1:10 dilution of soap nut solution for  $\frac{1}{2}$  hour. This suggests the bactericidal action of soap nut to some extent and also that bacteria are more sensitive to the treatment when not associated with alga.

We are grateful to Prof. J. J. Chinoy, Director, for the facilities. This work is a part of the research project supported by the Department of Atomic Energy, India.

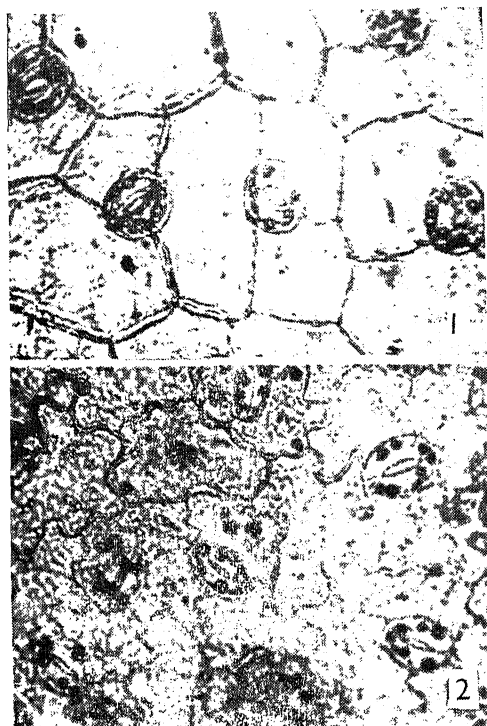
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### DEVELOPMENT OF SUBSIDIARY CELLS AND WATER LOSS IN *CROTALARIA* *MADICAGINEA* LAMK.

A KNOWLEDGE of water requirement of plants and their physical environment is necessary for understanding the adaptation of vegetation under stress conditions<sup>2</sup>. The present work shows the development of subsidiary cells as an adaptive feature and water loss in *Crotalaria medicaginea*, growing in Indian arid zone. Presence of two types of stomata in *C. medicaginea* has already been reported<sup>1</sup>. Stomata with and without subsidiary cells are present and the openings of the latter were less rhythmic than those of the former. Number of stomata with or without subsidiary cells per unit area decreases as the leaves mature (Figs. 1–2). In the early stages of leaf maturity, the number of stomata with subsidiary cells are less, but their proportionate numbers increase as the leaf attains maturity (Table I). Correlating these observations with water loss, the

percentage of water loss was more from leaves where the number of stomata without subsidiary cells was more. This may also be correlated with less cuticular thickening in young leaves but the main reason is the opening of stomata in young leaves which are without subsidiary cells.



FIGS. 1-2. Fig. 1. Epidermal peeling from the leaf of a seedling showing stomata without any subsidiary cells,  $\times 550$ . Fig. 2. Epidermal peeling from a grown up plant showing stomata with subsidiary cells having distinct globules,  $\times 550$ .

TABLE I

Differently matured leaves, having stomatal percentage with and without subsidiary cells, number of stomata per unit area and the percentage of water loss in 30 mts duration in *C. medicaginea*\*

| State of maturity of leaves | % of water loss | Number of stomata (mm sq.) | % of stomata with sub. cell | % of stomata without sub. cell |
|-----------------------------|-----------------|----------------------------|-----------------------------|--------------------------------|
| Young                       | 17.0            | 424                        | 18.0                        | 82.0                           |
| Mature                      | 14.6            | 243                        | 53                          | 47                             |
| Old                         | 13.5            | 176                        | 78                          | 22                             |

\* Average of three samples.

It may, therefore, be concluded that more number of subsidiary cells develop as an adaptive feature to check water loss enabling plants to grow successfully in arid conditions.

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#### IMPROVING PHOTOSYNTHETIC AREA AND ENERGY CONSERVING EFFICIENCY OF WHEAT BY GIBBERELIC ACID

ALL the green plant parts, trap and conserve the solar energy. The vertically standing photosynthetic parts conserve more energy than the horizontally standing green parts<sup>1</sup>, and hence the high ratio of vertical/horizontal green tissues may indicate the fixation of more solar energy. Keeping this in mind, gibberellin ( $GA_3$ ) which increases the length of internode and leaf sheath<sup>2</sup>, was used for changing the ratio of vertical/horizontal green tissues in wheat. Effect of gibberellic acid on energy conservation and dry matter production has also been recorded.

Wheat (Kalyansona) seeds were sown @ 90 kg/ha on half hectare area after normal dose of fertilizer application (90 N, 50  $P_2O_5$  and 50  $K_2O$  kg/ha) to the soil. Gibberellic acid at 100 ppm level was sprayed on the 15 day old plants in 5 plots each of  $5 \times 5$  meter in size. The area of leaves and leaf sheath were calculated from the length and width of leaves using Kemp's<sup>3</sup> formula and surface area of ear was calculated following the method of Teare and Peterson<sup>4</sup>. The angle of leaf that subtended between the horizontal and two-third portion of leaf which showed a uniform angle, was taken as standing angle of leaf. The average chlorophyll of photosynthetic parts was determined by the method of Arnon's<sup>5</sup>. The rate of  $CO_2$  uptake and release during photosynthesis and respiration of whole plant was measured in triplicate over half an hour period at 6, 8, 10, 12, 14 and 18 hours during a day, following the technique devised by Dwivedi<sup>6-7</sup>. The amount of  $CO_2$  assimilated was converted into energy value (cal.) on the basis of usual formula of photosynthesis<sup>1-7</sup>. The value of the energy

TABLE I

Effect of GA on the photosynthetic area index, ratio of vertical/horizontal green tissues (V/H green tissue), chlorophyll content of photosynthetic parts and energy trapping and conserving efficiency of wheat

| Age of plants | Treatments | Photo-area index | Ratio of V/H green tissue | Chlorophyll (a+b) (mg m <sup>2</sup> land area) | Energy efficiency (%) |                  |                  |
|---------------|------------|------------------|---------------------------|---|-----------------------|------------------|------------------|
|               |            |                  |                           |   | Trapping              | Conserving       |                  |
|               |            |                  |                           |   |                       | CAM <sup>+</sup> | HM <sup>++</sup> |
| 28            | Control    | 0.28             | 0.02                      | 96.0  | 1.48                  | 1.21             | 1.03             |
|               | GA 100 ppm | 0.30             | 0.2                       | 97.0  | 1.53                  | 1.22             | 1.06             |
|               | 't' value  | 1.3              | 3.7*                      | 2.0   | 1.60                  | 0.20             | 0.60             |
| 56            | Control    | 8.8              | 0.04                      | 716.8   | 3.60                  | 3.15             | 2.58             |
|               | GA 100 ppm | 9.1              | 0.3                       | 718.9   | 5.04                  | 4.50             | 3.90             |
|               | 't' value  | 1.8              | 2.8*                      | 2.4   | 2.61*                 | 2.71*            | 2.80*            |
| 84            | Control    | 8.6              | 0.04                      | 880.2   | 3.91                  | 3.29             | 2.64             |
|               | GA 100 ppm | 9.0              | 0.4                       | 882.5   | 5.31                  | 4.51             | 3.99             |
|               | 't' value  | 2.0              | 3.8*                      | 2.6   | 2.70*                 | 2.89*            | 2.72*            |
| 112           | Control    | 7.6              | 0.06                      | 373.2   | 1.34                  | 0.78             | 0.51             |
|               | GA 100 ppm | 8.3              | 0.5                       | 374.0   | 3.97                  | 3.14             | 2.89             |
|               | 't' value  | 1.7              | 3.2*                      | 1.9   | 3.44*                 | 3.12*            | 3.22*            |
| 140           | Control    | 4.8              | 0.1                       | 62.1  | 0.52                  | ..               | ..               |
|               | GA 100 ppm | 5.0              | 0.6                       | 63.4  | 0.70                  | ..               | ..               |
|               | 't' value  | 1.3              | 2.8*                      | 1.9   | 2.91*                 | ..               | ..               |

\* 't' value significant at 5% level.

+ CAM—Carbon assimilation method;

++ HM—Harvest method.

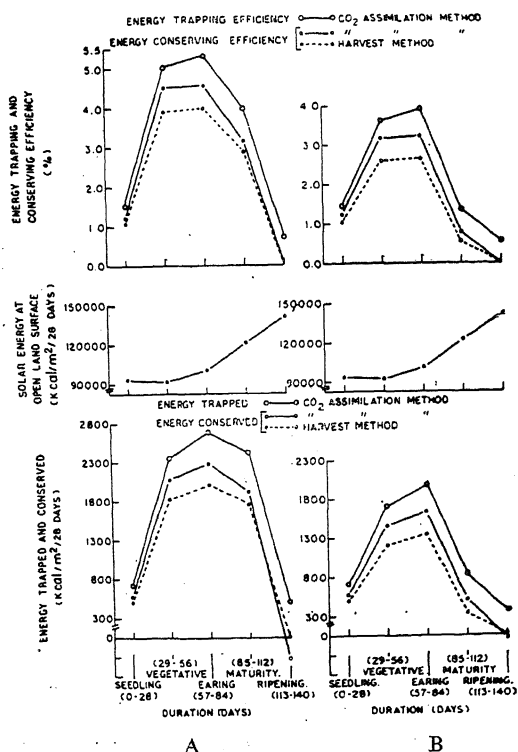


FIG. 1. Effect of GA on the photosynthetic capacity and energy trapping and conserving efficiency of wheat. A. GA 100 ppm. B. Control.

efficiency obtained by the CO<sub>2</sub> assimilation method was also compared with the harvest method<sup>7</sup> (Table I, Fig. 1). Solar radiation (cal./cm<sup>2</sup>/sec.) was measured by solarimeter and the efficiency of energy conservation was calculated by dividing the trapped and conserved energy during each growth stage by half the solar radiation of that stage. All these observations were recorded at the age of 28, 56, 84, 112 and 140 days of plant growth.

It is evident from Table I that the photosynthetic area index of plants increased by GA, though the variations were not significant. The ratio of vertical/horizontal green tissue was significantly higher in GA treated plants as compared to the control. The chlorophyll content of GA treated plants also did not increase over control significantly. However, CO<sub>2</sub> fixation rate and energy efficiency of plants were found to increase by 25% more than that of the untreated (Table I, Fig. 1). The increase in energy conserving efficiency due to GA treatment calculated by both the harvest and gas analysis method were also comparable (Table I, Fig. 1). Treharne and Stoddart<sup>8</sup> reported an increase in photosynthesis due to enhancement in RuDP carboxylase activity and rise in chlorophyll content in GA treated red clover plants. On the basis of the results of present investigation, the increase in ratio of vertical/horizontal green tissues may be suggested as one of the main reasons for rise in carbon assimilation rates of GA-treated wheat plants.

Thanks are due to Prof. R. Misra for guidance and providing the laboratory facilities and also to Dr. O. S. Singh for giving valuable suggestions.

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### EFFECT OF CYCOCEL ON SEX EXPRESSION ON SOME CUCURBITACIOUS PLANTS

THE sex expression in plant is genetically controlled but various chemicals are reported to modify this phenomenon. Gibberellic acid is known to retard pistillate appearance<sup>1</sup> and enhance staminate differentiation<sup>2</sup>. Growth retardants have been shown to increase femaleness<sup>3,4,5</sup>. Members of family Cucurbitaceae exhibit a wide diversity of sex forms and cycocel is a growth retardant. The effect of cycocel was studied on sex expression in six species of this family.

The experiment was conducted at IARI Plant Introduction Station, Jodhpur, during the summer, 1970. Six members, viz., squash melon (*Citrullus vulgaris* Schrad. var. *fistulosus* Duth. and Full.), ridge gourd [*Luffa acutangula* (L.) Roxb.], cucumber (*Cucumis melo* L. var. *utilissimus* Duth. and Full.), musk melon (*Cucumis melo* L.), water melon (*Citrullus vulgaris* Schard. ex Eckl. and Zeyh.) and bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] were sown at 2.0 × 1.0 metre spacing. Three concentrations of Cycocel; viz., 500 ppm, 1,000 ppm and 2,000 ppm were sprayed at two, four and eight leaf stages of seedlings. Five plants in each treatment were tagged and sex ratio in each species was determined.

The data revealed that cycocel treatment induced femaleness by increasing the number of pistillate flowers and proportionately reduced the number of staminate flowers. This confirms the findings of Halevy and Rudich<sup>3</sup> (using B-995 in musk melon,

Bose and Ghose<sup>4</sup> (using B-9 in cucurbits) and Iwahori *et al.*<sup>5</sup> (using 2-chloroethane phosphonic acid in cucumbers). In the present case where cycocel has been used, the effect was more pronounced in bottle gourd which normally did not bear any pistillate flowers. In general 1,000 ppm was more effective as compared to 500 ppm and 2,000 ppm, except in the case of squash melon and cucumber where 2,000 ppm induced more femaleness (Table I).

TABLE I

Effect of cycocel on sex ratio (staminate/pistillate)  
in six species of family Cucurbitaceae

| Species      | Control           | 500 ppm | 1,000 ppm | 2,000 ppm |
|--------------|-------------------|---------|-----------|-----------|
| Squash melon | 5.4               | 1.9     | 2.7       | 1.8       |
| Ridge gourd  | 17.7              | 3.2     | 1.6       | 3.3       |
| Cucumber     | 13.9              | 2.8     | 3.0       | 2.6       |
| Water melon  | 11.6              | 3.2     | 3.3       | 3.2       |
| Musk melon   | 17.0              | 2.4     | 2.3       | 2.5       |
| Bottle gourd | Only<br>Staminate | 3.1     | 2.3       | 2.7       |

It is also noteworthy that the treated plants of all the species were shorter due to reduction of internode length, had thick and dark green leaves, smaller tendrils and more hairs, as noted by Iwahori *et al.*<sup>5</sup> with 2 chloroethane phosphonic acid.

Thanks are due to late Dr. H. B. Singh, Head, Division of Plant Introduction, for providing facilities and to Dr. S. D. Sharma, Botanist (Rice), IARI, Regional Research Station, Hyderabad, for preparation of the manuscript.

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# ARAUCARIAN ROOTS FROM THE JURASSIC OF RAJMAHAL HILLS, INDIA

The Rajmahal Hills have yielded the largest variety of fossil plants known from the Jurassic rocks of India<sup>1-7</sup>. The major plant groups occurring in this area are ferns, cycads, Bennettitales, Pentoxyleae and conifers which are represented by stems, petioles, leaves, sporangia and seeds. No gymnospermous roots are known and the only roots described from the beds are those found in association with some of the filician rhizomes like *Tinipaharia sinuosa* Jacob<sup>3</sup>, *Osmundacaulis sahnii* (Mittre)<sup>4</sup> and *O. rajmahalensis* (Gupta)<sup>2</sup>. However, in some material collected by the senior author (BDS) from a newly discovered locality of Pakur in the Santhal Pargana District of Bihar, petrifications of isolated roots of ferns and conifers are frequently found. The locality is situated 4 km North West of the railway station Pakur. The fossiliferous rock is yellowish-white and rests on a thick layer of trap which is made up of black, hexangular pillars.

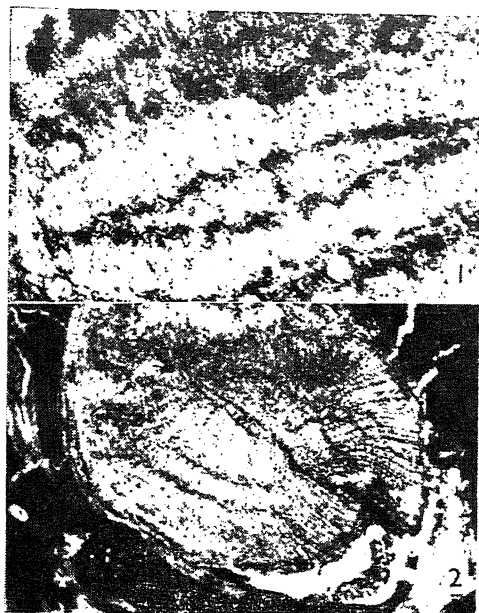
The roots are 3-12 mm in diameter with two exarch protoxylem points (Fig. 1). The primary xylem is made up of hexagonal tracheids while those of the secondary xylem are narrower and rectangular in shape. Even a thin root (4.5 mm diameter) may show well developed secondary growth (Fig. 2). Xylem parenchyma and resin canals are absent. In older roots the secondary xylem on the two sides of the primary xylem plate may be equally or unequally developed and the primary xylem may become obscure (Fig. 2).

Wood rays are 1-3 cells high, uniseriate and homogeneous. Tangential walls of tracheids are smooth while the radial walls are provided with uni to triseriate, contiguous bordered pits. Pits in cross fields are not visible.

Phloem is radial and made up of thin walled cells filled with some dark staining matter (Fig. 1). The secondary phloem in the majority of the roots is represented by an unpreserved narrow zone outside the secondary xylem. Cortex is 0.6-0.9 mm wide, parenchymatous and provided with numerous, hexagonal scleroids. In the outer portion of cortex a thick layer of periderm is seen in older roots which is made up of radially arranged, thin walled, narrow cells in 6-10 lines.

In the nature of secondary xylem the present material shows the typical characters of Araucariaceae, i.e., compact wood without resin ducts, tracheids having continuous bordered pits on their radial walls and presence of uniseriate, small wood

rays<sup>6</sup>. The occurrence of araucarian stems and megastrobili in the new locality may lend some support to the araucarian affinities of these roots.



FIGS. 1-2. Fig. 1. A young root, showing diarch xylem,  $\times 36$ . Fig. 2. An old root showing compact secondary xylem on two sides, the primary xylem is not seen,  $\times 12$ .

Comparison was also made with the cupressoid roots described from the Jurassic of Arctic<sup>3</sup>, and other known coniferous roots, but did not find fruitful.

The present study is interesting as it is the first description of a gymnospermous root from the Rajmahal Hills, India.

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## REVIEWS AND NOTICES OF BOOKS

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Indian Dairy Products. By K. S. Rangappa and K. T. Achaya. (Asia Publishing House, Bombay-1), 1974. Pp. xiii + 386. Price Rs. 40-00.

This is a book mainly devoted to the Indian dairy products. The first edition *The Chemistry and Manufacture of Indian Dairy Products* was published in 1948. The present one is a revised edition incorporating considerable amount of work that has been carried out at various centres for the past two decades. The book is divided into 3 parts. Part 1 consists of milk and unfermented milk products. Part 2 comprises of information in fermented milk products. Information on ghee is well covered in Part 3.

Under Part 1, the subject has been dealt with under 7 chapters covering the production, composition and properties of milk, the major and the minor constituents of milk, the variations in the composition besides adulteration and legal standards. The bacteriological aspect and effect of heat treatment and the unfermented milk products also have been included.

There are three chapters in Part 2 covering fermentation in Indian Dairy Industry, preparation, quality and composition of dahi, lassi and butter.

The various aspects such as ghee and its place in the Indian diet, attributes, preparation and grading of ghee, constituents of ghee, the analytical characteristics of ghee, the adulteration of ghee and its detection and rancidity in ghee are all well brought out in Part 3.

The book is well written. The subject-matter has been dealt with precisely and concisely. The references to the literature have been exhaustive. The material has been well presented and the authors deserve congratulations.

This is a very good reference book which should find a place in the dairy, food and chemical industry, as well as technology and academic institutions.

It would have been ideal and useful had the authors taken a little more pains in including latest statistical data pertaining to cattle census, production and utilization of milk and milk products and the like.

C. P. A.

for the first time that such an attempt has been made. Dairying is very much a part of the general agricultural farming which is reflected in the low intensity of milk production. Indian dairy industry has several novel and interesting features. The yield per animal in most cases is below economic standard when an animal would give a return to the owner. This is the result of poor nutrition of the animals on account of small farm holdings, limited irrigation facilities, and severe climate for a substantial part of the year. Dairy animals in most cases subsist on the coarsest of feeds (*Kadbi*, straws). Over 70% of the total milk production is contributed by small farms (below 2 hectares) and landless labourers. The producer adopts simple but sound technology to convert even small surpluses into ghee, *khoa* and such other products which are in wide demand. Milk has a free market, the quality being adjusted to the price one pays and balanced against the prevailing price of products. In this traditional pattern of dairying, a new approach has been introduced, during the past 50 years, with coming into the market, products like table butter, cheese, milk powder, etc. The author has summarised the developments leading to the growth of the industry under Five-Year Plans. Almost every urban centre has now a milk processing plant for the supply of pasteurised bottled milk. In contrast, did we not have even ten establishments in the country till the year 1945, for the supply of pasteurised milk in a sizable quantity.

The author has been closely associated in pioneering various developments, the most notable being the organisation of the milk supply scheme for Bombay. Though the book was prepared with a view to providing a handy manual to the delegates participating in the XIX International Dairy Congress held in New Delhi in December 1974, it is of wider interest and will be useful as a permanent reference book to all associated with the industry. The contents have been lucidly presented and a lay-reader would benefit by the opportunity offered by the book to acquaint with a topic of perennial interest. The author has drawn liberally on his wide experience and associations in India and abroad, to bring to light not only technical matters but also interesting side information. '*Pinjrapole*' as an institution conjures thoughts of providing protection and care for cattle. It is, thus, interesting to learn that the Pinjrapole in Bombay was established by the merchant community to protect stray dogs. The

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Dairying in India—A Review: D. N. Khurody, Asia Publishing House, Bombay 400 038, 1974. Pp. xiii + 255. Price Rs. 60-00.

The book gives a consolidated review of the development of the dairy industry in India. It is

gap between the requirements and supplies of milk has widened in the past two decades. The urban supplies have come to depend more and more on skim milk powder and butter-oil to fulfil the demand for cheap milk. Hardly 5% of the milk produced is handled by milk schemes and for manufacturing western type of dairy products. Most of the development funds come to be concentrated for the benefit of a small segment of the populace. In the same way, though the country derives about 60% of milk supplies from the buffalo, little is done towards its development. Even to-day a large volume of milk is utilized for the manufacture of indigenous products but little attention has been paid for their improvement and development. The author has touched these points in detail and given valuable suggestions for the progress of the dairy industry which to-day contributes about half of the total agricultural income, and a quarter of the net national income of India.

Besides the background information, the book has been divided into fifteen sections and is well illustrated with 66 full-page photographs and statistics. Breeds of dairy animals and current topics like cow protection, place of the buffalo in the dairy industry, cross-breeding of cattle with exotic animals, quality of milk and milk products, and the scope for reducing overheads in dairy operations have been discussed. Separate chapters describe cattle fairs, foreign aid received by the dairy industry, dairy education and research, dairy machinery and equipment industry, and organizations engaged in dairy development. Subject-index is also given.

The book has been clearly printed on semi-art paper and is attractively bound.

N. N. D.

**A Dictionary of Flowering Plants in India.**  
By H. Santapau and A. N. Henry. (Published by the Publications and Information Directorate, CSIR, Hillside Road, New Delhi-12), 1973. Pp. vii + 198. Price : India : Rs. 22-00 ; Foreign : £ 3-50 or \$ 9.00.

This dictionary written by two leading taxonomists gives brief descriptions of 2,890 genera belonging to 328 families of flowering plants in India. The generic names are alphabetically arranged with their respective families in parentheses. For every genus the total number of species found in the world as well as in India are listed and its habit

is described. Some of the more common species occurring in India, well-established local or regional name(s) of the species and their economic uses, if any, are given. This publication should find a place in every library as ready reference for students of taxonomy and others who are interested in botanical studies.

G. S. R.

## ANNOUNCEMENTS

### Institution of Chemists (India) Associateship Examination, 1976

The Twenty-sixth Associateship Examination of the Institution of Chemists (India) will be held in November, 1976. The last date for Registration is 30th November, 1975. The Examination in Group A (Analytical Chemistry) is divided into eleven sections and each candidate will be examined in two of them. In addition to the General Chemistry, Organic, Inorganic, Physical and Applied Analytical Chemistry form the subjects of General Chemistry. The Examination is recognised by the Government of India as equivalent to M.Sc. in Chemistry for purposes of recruitment of Chemists. Further enquiries regarding this and for Membership may be made to the Honorary Secretary, Institution of Chemists (India), Chemical Department, Medical College, Calcutta-12.

### Nuclear Physics and Solid State Physics Symposium, 1975

The above symposium, organized under the auspices of the Department of Atomic Energy, will be held this year at the Variable Energy Cyclotron Project, Calcutta, during December 22-26, 1975. The deadline date for receipt of abstracts is October 20, 1975.

Detailed information can be obtained on request from Dr. R. Subramanian, Convener, NP and SSP Symposium Committee, Nuclear Physics Division, Bhabha Atomic Research Centre, Bombay 400085.

### Computer Society of India, Bombay

The Computer Society of India (CSI) is holding its next Annual Convention of Computers and Social Change at Hyderabad, on January 20-23, 1976.

For details please write to : DVR Vithal, Program Chairman, CSI 76, Tata Institute of Fundamental Research, Bombay 400005.

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## ABSTRACT

The essential oil of *Laggera aurita* on extensive application of chromatographic technique afforded  $C_{27}$ (I),  $C_{32}$ (II) alkanes, together with  $\delta$ -cadinene, 2, 3-dimethoxy-*p*-cymene (a new aromatic ether), laggerol (a new sesquiterpenic secondary alcohol),  $\alpha$ -cadinol and *m*-menth-6-en-8-ol. The identities of these compounds have been ascertained on the basis of their physical, chemical and spectral data.

THE plant *Laggera aurita*<sup>1</sup> is an annual shrub growing throughout Madhya Pradesh. In Malwa region it is known as 'Kukronda'. It flowers during December to February and bears pink coloured flowers. A survey of literature shows that no work has been done on the chemical examination of the steam volatile constituents of the plant. In the present work, the whole plant at full flowering stage was subjected to steam distillation which gave a dark yellow oil (0.04%) with a characteristic sweet smell. The oil shows the presence of nine components on TLC as well as on GLC examination, seven of them have been identified as shown in the abstract.

## EXPERIMENTAL

The essential oil was found to have the following physical and chemical constants:  $n_D^{26}$ , 1.498;  $d_4^{26}$ , 1.0742; ( $\alpha$ )<sub>D</sub><sup>26</sup>,  $\pm 0$ ; acid value, 0.76; ester value, 5.23 and boiling range, 54–114°/1 mm.

The oil was separated into acidic and neutral parts by washing with sodium hydroxide solution (5%). No work was possible on the acidic portion due to paucity of the material. The neutral portion (10 g) was chromatographed over neutral alumina grade II (1 : 30) and three major fractions were collected by eluting the column with petroleum ether, benzene and ether.

Petroleum ether fraction (3.5 g) was again chromatographed over active silica-gel followed by silica-gel impregnated with silver nitrate (15%) which gave three compounds A, B, and C in TLC pure form.

**Compound 'A'**, b.p. 170–172°/13 mm;  $n_D^{21}$ , 1.432 analysed for  $C_{27}H_{56}$ . This was crystallised from chloroform-methanol as colourless plates (0.24 g), m.p. 60–61°.  $\nu_{\max}$ , 2900, 1460, 1350, 1020, 840, 732 and 722  $cm^{-1}$  and the NMR signals at 8.70 and 8.20  $\tau$ . The compound 'A' was characterised as *n*-heptacosane<sup>2,3</sup> by comparison of its IR and NMR spectra with that of an authentic sample.

**Compound 'B'** b.p. 240°/13 mm.  $n_D^{27}$ , 1.442 analysed for  $C_{32}H_{66}$ . This was crystallised from chloroform-methanol as colourless plates (0.13 g), m.p. 71–72°.  $\nu_{\max}$ , 2950, 1470, 1385, 1038, and

722  $cm^{-1}$  and NMR signals at 8.70 and 8.20  $\tau$ . It was characterised as *n*-dotriacontane<sup>4,5</sup> by comparison of its IR and NMR spectra with that of an authentic sample.

**Compound 'C'**, b.p. 125–127°/2 mm;  $n_D^{29}$ , 1.502 analysed for  $C_{15}H_{24}$ .  $\nu_{\max}$ , 837 (trisubstituted double bond), 1385 and 1370 (isopropyl group), and 2940 and 1450  $cm^{-1}$  (C–H and C–CH<sub>3</sub>). The IR spectrum of this compound is superimposable with that of  $\delta$ -cadinene<sup>6,7</sup>. The identity was confirmed through catalytic dehydrogenation, selenium dehydrogenation and ozonolysis.

**Benzene fraction** (2.3 g) was chromatographed over silica-gel impregnated with silver nitrate (15%), which gave two compounds 'D' and 'E' in TLC pure form.

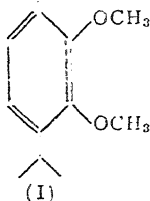
**Compound 'D'** was distilled under reduced pressure, b.p. 97–98°/0.5 mm;  $n_D^{29}$ , 1.486; ( $\alpha$ )<sub>D</sub><sup>29</sup>,  $\pm 0$  analysed for  $C_{12}H_{18}O_2$  with 2 (OCH<sub>3</sub>)<sub>2</sub> groups (M, m/e : 154).  $\nu_{\max}$ , 2986 (C–H), 1379, 1362 and 1178 (isopropyl group), 1210, 1157 and 1065 (phenolic ether), 850 (two free adjacent hydrogen atoms in the aromatic ring) and 805  $cm^{-1}$  (1 : 2 : 3 : 4 tetra substituted benzene)<sup>8</sup>. NMR (CCl<sub>4</sub>,  $\tau$ ), 8.88 and 8.76 (6 H, two methyls of isopropyl groups), 7.80 (3 H of one methyl group); 3.30 and 3.26 (2 H, aromatic); 6.18 (6 H of two methoxyl groups) and 6.90–6.40 (*m*, of one benzylic proton). On comparing the NMR spectrum with that of *p*-cymene, there was an agreement in all the signals except 6.18  $\tau$  (two methoxyl groups). This shows that compound 'D' has *p*-cymene skeleton with two methoxyl groups.

The  $\lambda_{\max}$  (log  $\epsilon$ ) of 'D' 228  $m\mu$  (3.91), 285  $m\mu$  (3.62) and the ratio of wavelength of the secondary to primary bands is fairly close to 1.22, which agrees with the presence of substituted benzene having –OCH<sub>3</sub> group.

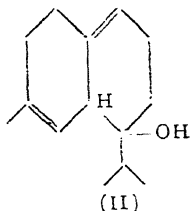
In the light of the aforesaid evidence, a tentative structure (I) has been proposed for this new ether and named as 2, 3-dimethoxy-*p*-cymene.

Uptill now only 2, 5-dimethoxy-*p*-cymene and 2, 5-dihydroxy-*p*-cymene<sup>9,10</sup> have been reported to occur in several essential oils but 2, 3-dimethoxy-

*p*-cymene has not yet been reported. However, it occurs in the essential oil of *Blumea membranacea* as presently reported by Joshi<sup>11</sup>.



Compound 'E', b.p. 109°/3.5 mm;  $n_D^{20}$ , 1.586;  $(\alpha)_D^{20}$ , -12.07° analysed for  $C_{17}H_{20}O$  ( $M$ ,  $m/e$ ; 222).  $\nu_{max}$ : 3430 (hydroxyl group), 1365, 1378, 1165 (isopropyl group), 890, 865 and 800 (trisubstituted double bond) and 2900  $cm^{-1}$  (C-H). NMR ( $CCl_4$ ,  $\tau$ ): 9.18, 9.12, 9.08 and 9.02 (2d, 6 H, 2  $CH_3$  of isopropyl group); 8.38 (S, 6 H, two methyl groups on two double bonds); 8.09 (broad S, three methylene groups conjugated to the double bonds); 4.79 (S, a proton on the trisubstituted double bond) and 7.72 (broad S, disappeared on  $D_2O$  exchange, hydroxyl group). The compound, therefore, seems to be a sesquiterpenic alcohol.



On selenium dehydrogenation in the presence of nitrogen, this compound gave cadalene in considerable yield which has been identified on the basis of melting point of its picrate. The formation of cadalene showed the presence of bisabolene skeleton in this compound. Therefore, the following tentative structure (II) has been proposed for it and named as Lagerrol.

Ether fraction (3.12 g) of neutral oil was chromatographed over silica-gel impregnated with silver nitrate (15%) which gave two compounds 'F' and 'G' in TLC pure form.

Compound 'F', b.p. 163–165°/3 mm; m.p. 70–71°;  $n_D^{20}$ , 1.4942;  $(\alpha)_D^{20}$ , -20.06° analysed for  $C_{15}H_{20}O$  and was identified as  $\alpha$ -cadinol<sup>12</sup> by IR, NMR and chemical evidence.

Compound 'G', b.p. 148°/8 mm;  $n_D^{20}$ , 1.471;  $(\alpha)_D^{20}$ , 29.40° analysed for  $C_{10}H_{16}O$  and was identified as *m*-menth-6-en-8-ol<sup>13</sup> by IR, NMR and chemical evidence.

#### ACKNOWLEDGEMENT

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## STUDIES ON QUINAZOLONES DERIVATIVES

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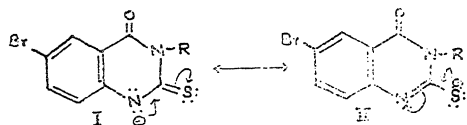
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THE discovery of various types of quinazolones as antimalarials<sup>1</sup>, CNS potent<sup>2</sup> and antibacterials<sup>3</sup> as well as their hydrazides as anti-inflammatory agents<sup>4</sup>

have created the interest of authors to prepare some 6-bromo-2-( $\beta$ -diethylaminoethylthio)-3-aryl (or alkyl)-4 (3 H) quinazolones and 6-bromo-2-carboethoxymethylthio-3-aryl-4 (3H)quinazolones as chemotherapeutical interest. The syntheses were carried out by the reaction of 6-bromo-3-aryl(or alkyl)-2-thio-4 (3 H) quinazolones with equivalent

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amount of  $\beta$ -diethylaminoethyl chloride and ethyl bromoacetate in 10% alcoholic NaOH solution at room temperature with occasional stirring for 2–3 hr. The reaction is simple and straightforward and completes in good yields. The two tautomeric structures of 6-bromo-2-thio-3-aryl-4(3H) quinazolones arising by the shift of proton and a pair of electrons may exist as resonance hybrids (I  $\leftrightarrow$  II). Therefore, in the alkylation, the entering group may become attached either to the nitrogen atom forming N-alkyl derivatives or to the sulphur atom giving thioether or a mixture of both.



The hydrolysis of alkylated product (8) with alcoholic hydrochloric acid gives the sulphur free compound 6-bromo-3-*p*-ethoxyphenyl-quinazoline-2,4-dione (3). The alkaline solution of mercaptan on treatment with lead acetate or silver nitrate gives characteristic coloured salts. The IR spectrum of compound (8) shows two characteristic absorption bands one at 1678  $\text{cm}^{-1}$  and another at 1650  $\text{cm}^{-1}$  for the exocyclic and cyclic (position-4) carbonyl groups respectively. But the IR spectrum of compound (3), as expected, shows the two absorption bands one at 1668  $\text{cm}^{-1}$  for the ring carbonyl group at position-4 and another at 1738  $\text{cm}^{-1}$  for the ring carbonyl group at position-2 along with a broad absorption band at 3245  $\text{cm}^{-1}$  for -NH bond. These evidences prove that the 6-bromo-2-thio-3-aryl-4(3H)quinazolones are more reactive in the thiol form (II) and are alkylated quantitatively on the sulphur atom rather than nitrogen atom. The structures of these quinazolones (Tables I and II) were also supported by their spectral as well as analytical data. The NMR spectrum of compound<sup>1</sup> shows along with other normal peaks, one doublet for  $\text{C}_5$ -proton due to long range coupling with  $\text{C}_7$ -proton at  $\delta$  8.45 ( $J = 2.0$  Hz). The  $\text{C}_7$ -proton appears as a pair of doublet or a quartet at  $\delta$  7.88; being doublet ( $J = 2.0$  Hz) due to long range coupling with  $\text{C}_5$ -proton and double doublet ( $J = 9.0$  Hz) due to coupling with adjacent  $\text{C}_6$ -proton. The IR spectrum, as expected, does not show any absorption in -NH region characteristic of starting material.

#### EXPERIMENTAL

The melting points of the compounds were recorded on GALLENCAMP Melting Point apparatus and are uncorrected. The compounds were chromatographed on developing the TLC plates in suitable solvents using silica gel (BDH) as adsorbent

and  $R_f$  values were recorded. Varian A60D model was used for recording of NMR spectra, a Perkin-Elmer 257 for IR and a Coleman Analyzer for analyses.

**6-Bromo-2-( $\beta$ -diethylaminoethylthio)-3-*p*-tolyl-4(3H)quinazolone (1).**—6-Bromo-2-thio-3-*p*-tolyl-4(3H)quinazolone<sup>5</sup> (2.1 g) was dissolved in the minimum quantity of 10% alcoholic NaOH solution and to this was added  $\beta$ -diethylaminoethyl chloride (1.0 ml). The reaction mixture was stirred and allowed to stand for about two hours at room temperature, when crystals separated out. They were filtered, washed with water and then with a little of alcohol. Recrystallisation from 80% ethanol afforded the needles, yield 78%, m.p. 106°. TLC:  $R_f = 0.70$  (Benzene-Ether, 3 : 1). Anal. Calcd for  $\text{C}_{21}\text{H}_{24}\text{N}_2\text{S}$ : OSBr: N, 9.41; S, 7.17. Found: N, 9.23; S, 6.73%. IR  $\nu_{\text{max}}^{\text{nujol}}$   $\text{cm}^{-1}$ : 1725s, 1680s, 1605m, 1550s, 1535m. NMR( $\text{CDCl}_3$ )  $\delta$  ( $J = \text{Hz}$ ): 8.45- (1 H, d,  $J = 2.0$ ), 7.93 (1 H, q,  $J = 2.0$  and 9.0), 7.45 (5H, m), 2.46 (3H, s), 2.54 (2H, m), 3.50 (8H, m), 2.65 (4H, q,  $J = 7.0$ ) and 1.11 (6H, m). Likewise, other quinazolones were prepared. Their structures, melting points and the purity of the compounds are listed as in Table I.

**6-Bromo-2-carboethoxymethylthio-3-*p*-chlorophenyl-4(3H)quinazolone (2).**—Ethyl chloroacetate (1.0 ml) was added to a solution of 6-bromo-2-thio-3-*p*-chlorophenyl-4(3H)quinazolone (2.2 g) dissolved in 5% alcoholic NaOH solution and the mixture was stirred for 6–8 hr at room temperature. It was acidified with 5% HCl solution. The crude mass thus obtained was regenerated by dissolving in 5%  $\text{NaHCO}_3$  solution and precipitated with 5% -HCl solution. It was further crystallised from alcohol, yield 66%, m.p. 197°. TLC:  $R_f = 0.40$  (Benzene-Ether, 12 : 1). Anal. Calcd. for  $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_3\text{SBr}$ : N, 6.18; S, 7.06. Found: N, 6.03; S, 7.20. IR  $\nu_{\text{max}}^{\text{nujol}}$   $\text{cm}^{-1}$ : 1730s, 1700s, 1610s, 1585s, 1550s.

Following the same procedure, other derivatives were prepared and listed as in Table II.

**Hydrolysis of 6-Bromo-2- $\beta$ -diethylaminoethylthio-3-*p*-ethoxy-4(3H)quinazolone (8).**—A mixture of 6-bromo-2- $\beta$ -diethylaminoethylthio-3-*p*-ethoxyphenyl-4(3H)quinazolone (8) (2.40 g), 6N-HCl (25 ml) and ethanol (30 ml) was refluxed on a water-bath at 80–90° for 6–8 hr. On cooling, the crystalline product was separated out. It was washed with water and finally with a little of ethanol. Crystallisation from chlorobenzene and ethanol mixture gave the product 6-bromo-3-*p*-methoxyphenyl-quinazoline-2,4-dione (3) yield, 64%, m.p. 287°. Anal. Calcd. for  $\text{C}_{16}\text{H}_{13}\text{N}_2\text{O}_3\text{Br}$ : N, 7.75. Found: N, 7.58%. IR  $\nu_{\text{max}}^{\text{nujol}}$   $\text{cm}^{-1}$ : 3245 broad, 1738s, 1668s, 1620s, 1605s and 1500m.



TABLE I

*Physical data and IR peaks of 6-bromo-2-( $\beta$ -diethylaminoethylthio)-3-aryl (or alkyl)-4(3H)quinazolones*

| Comp. No.                 | Substituent R | Molecular formula   | Yield (%) | M.P. (°C) | Nitrogen (%) |        | Sulphur (%) |        | Characteristic IR peaks (cm <sup>-1</sup> ) | R <sub>f</sub> * values |
|---------------------------|---------------|---|-----------|-----------|--------------|--------|-------------|--------|---|-------------------------|
|                           |               |   |           |           | Found        | Calcd. | Found       | Calcd. |   |                         |
| 4. Phenyl                 |               | C <sub>20</sub> H <sub>22</sub> N <sub>3</sub> OSBr               | 48        | 145       | 9.48         | 9.72   | 7.29        | 7.40   | 1695s, 1605m, 1550s, 1515m                  | 0.68                    |
| 5. <i>p</i> -Chlorophenyl |               | C <sub>20</sub> H <sub>21</sub> N <sub>3</sub> OSClBr             | 85        | 310       | 8.85         | 9.00   | 6.37        | 6.85   | ..  | 0.75                    |
| 6. <i>p</i> -Bromophenyl  |               | C <sub>20</sub> H <sub>21</sub> N <sub>3</sub> OSBr <sub>2</sub>  | 91        | 149       | 7.67         | 8.21   | 6.02        | 6.26   | 1695s, 1645w, 1565w, 1545s                  | 0.65                    |
| 7. Benzyl                 |               | C <sub>21</sub> H <sub>24</sub> N <sub>3</sub> OSBr               | 59        | 250       | 9.06         | 9.41   | 6.58        | 7.17   | ..  | 0.63                    |
| 8. <i>p</i> -Ethoxyphenyl |               | C <sub>22</sub> H <sub>24</sub> N <sub>3</sub> O <sub>2</sub> SBr | 73        | 216       | 8.82         | 8.86   | 6.85        | 6.73   | 1678s, 1650s, 1560s, 1550s                  | 0.72                    |

\* R<sub>f</sub> values were measured on developing the TLC plates (adsorbent, silica gel BDH) in benzene-ether (3:1) mixture.

TABLE II

*Physical data and IR peaks of 6-bromo-3-aryl-2-carboethoxymethylthio-4(3H)quinazolones*

| Comp. No.                   | Substituent R | Molecular formula  | Yield (%) | M.P. (°C) | Nitrogen (%) |        | Sulphur (%) |        | Characteristic IR peaks (cm <sup>-1</sup> ) | R <sub>f</sub> * values |
|-----------------------------|---------------|--|-----------|-----------|--------------|--------|-------------|--------|---|-------------------------|
|                             |               |  |           |           | Found        | Calcd. | Found       | Calcd. |   |                         |
| 9. Phenyl                   |               | C <sub>18</sub> H <sub>15</sub> N <sub>2</sub> O <sub>3</sub> SBr              | 68        | 310       | 6.62         | 6.68   | 7.68        | 7.64   | 1725s, 1675s, 1590s, 1560s                  | 0.43                    |
| 10. <i>o</i> -Tolyl         |               | C <sub>19</sub> H <sub>17</sub> N <sub>2</sub> O <sub>3</sub> SBr              | 52        | 265       | 6.13         | 6.46   | 7.25        | 7.39   | ..  | 0.38                    |
| 11. <i>m</i> -Tolyl         |               | C <sub>19</sub> H <sub>17</sub> N <sub>2</sub> O <sub>3</sub> SBr              | 59        | 308       | 6.25         | 6.46   | 7.48        | 7.39   | ..  | 0.25                    |
| 12. <i>p</i> -Tolyl         |               | C <sub>19</sub> H <sub>17</sub> N <sub>2</sub> O <sub>3</sub> SBr              | 72        | 245       | 6.35         | 6.46   | 7.42        | 7.39   | 1730s, 1675s, 1600m, 1570s                  | 0.28                    |
| 13. <i>p</i> -Bromophenyl   |               | C <sub>19</sub> H <sub>15</sub> N <sub>2</sub> O <sub>3</sub> SBr <sub>2</sub> | 67        | 206       | 5.45         | 5.62   | 6.61        | 6.42   | ..  | 0.35                    |
| 14. <i>o</i> -Methoxyphenyl |               | C <sub>19</sub> H <sub>17</sub> N <sub>2</sub> O <sub>4</sub> SBr              | 54        | 280       | 6.10         | 6.23   | 7.35        | 7.11   | ..  | 0.20                    |
| 15. <i>p</i> -Methoxyphenyl |               | C <sub>19</sub> H <sub>17</sub> N <sub>2</sub> O <sub>4</sub> SBr              | 74        | 222       | 6.19         | 6.23   | 7.02        | 7.11   | 1725s, 1680s, 1610m, 1565s                  | 0.27                    |
| 16. <i>p</i> -Ethoxyphenyl  |               | C <sub>20</sub> H <sub>19</sub> N <sub>2</sub> O <sub>4</sub> SBr              | 65        | 266       | 5.93         | 6.04   | 7.15        | 6.91   | 1735s, 1680s, 1615m, 1565s                  | 0.23                    |

\* R<sub>f</sub> values were measured on developing the TLC plates (adsorbent silica gel) in benzene-ether (3:1) mixture.

The screening test of these compounds is in progress and will be reported in due course.

## ACKNOWLEDGEMENT

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## INTRACELLULAR CHARGES AND REGULATION OF PHOSPHORYLASE ACTIVITY IN THE GASTROCNEMIUS MUSCLE OF FROG

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### ABSTRACT

The levels of protein, glycogen, phosphorylases 'a' and 'b' have been investigated in the control, cathodal and anodal halves of the gastrocnemius muscle of the frog. The cathodal half contains high protein and phosphorylase activity as compared with control and anodal halves. Glycogen content does not show significant change between the control and experimental halves. The elevated activity of phosphorylases is attributed to the altered levels of calcium ions and adenine nucleotides in the respective muscle halves.

**P**REVIOUS investigations in this laboratory on subcellular electrokinetics in muscle cells have shown that the major cell contents in general possess a net positive charge at physiological pH, and when subjected to a field of direct current tend to show cathodal migration<sup>1,2</sup>. In addition, the control and experimental muscle halves are known to have distinct and different metabolic potentialities<sup>3</sup>. In view of this, it was considered necessary to gain some information on the glycogen metabolism of the muscle subjected to subcellular electromigration. A preliminary communication has been presented<sup>4</sup>.

### MATERIAL AND METHODS

The two gastrocnemius muscles of *Rana hexadactyla* were isolated with least injury from a freshly pithed animal. One of the pair was subjected to subcellular electromigration by exposing to long axis voltage gradient for 15 min. The control, cathodal and anodal halves were obtained as described previously<sup>1</sup>.

Glycogen was estimated<sup>5</sup> from the control and the muscle halves subjected to electromigration. The activities of phosphorylases 'a' and 'b' were estimated in the direction of glycogen synthesis<sup>6</sup>.

A 5% homogenate was prepared in aqueous medium containing 0.037 M ethylene diamine tetra acetic acid (EDTA), pH 6.5 and 0.1 M sodium fluoride, pH 6.5, as recommended by Guillory and Mommaerts<sup>7</sup>. After centrifugation for 15 min at 1000 × g, the supernatant was diluted four times with cysteine (0.03 M)-β-glycerophosphate (0.015 M) buffer, pH 6.5. The diluted enzyme (0.4 ml) was added to 0.2 ml of 2% glycogen and incubated for 20 min at 35° C. The reaction was started by the addition of 0.2 ml of 0.016 M glucose-1-phosphate (G-1-P) to one tube (phosphorylase 'a'), 0.2 ml of G-1-P and 0.004 M adenosine '5-monophosphate to the other (phosphorylase 'b'). After incubation for 15 min. for phosphorylase-b, and 30 min for phosphorylase 'a' activities, the reaction was stopped by the addition of 10% sulphuric acid. Inorganic phosphate (Pi)

liberated was estimated<sup>8</sup>. Phosphorylase activity was expressed as μ moles of Pi liberated/mg protein/hr. Protein was estimated by Biuret method<sup>8</sup>.

### RESULTS AND DISCUSSION

Average values of soluble proteins in the control muscles (C 1 and C 2) are given in Table I and they were found to be similar. The soluble protein content is different in the two halves of the experimental muscle (Table I). There is no difference in the protein contents of the cathodal half and the control, whereas the anodal half had 40.1% less protein compared to the cathodal half. The difference in the distribution between the KH and the AH is attributed to a net positive charge density of the major sarcoplasmic proteins and their consequent cathodal migration<sup>10-13</sup>.

Glycogen content also shows variation in its level in the experimental halves, even though the difference is not statistically significant. Since glycogen does not possess any charge, the change in its level may not be due to electromigration. Glycogen is a rapidly mobilizable and labile energy fuel and it is possible that there may be a passive mobilization from the anodal to cathodal half due to increased energy demands in the cathodal half<sup>10-13</sup>.

The activities of phosphorylase 'a', as well as 'b' also showed variations in the experimental halves (Table I). The difference in the activity of phosphorylase 'a' between KH and AH (43.2%) is significant. A similar trend was observed for phosphorylase 'b' also. It is of interest to note that the elevated glycogen content and phosphorylase activity in the KH as compared to AH are similar to that of white (fast) muscle which has higher glycogen content and glycogenolysis<sup>14</sup>.

Phosphorylase kinase, a key enzyme known for its regulation of the activity of phosphorylase and consequent glycogenolysis, is known to be calcium (Ca<sup>++</sup>) dependent<sup>15,16</sup>. Elevated levels of Ca<sup>++</sup> activate this kinase which in turn converts phosphorylase 'b' to 'a'. In the present study the KH has higher activity of phosphorylase 'a'. This

TABLE I

The levels of proteins (mg/gm wet weight), glycogen (mg/gm wet weight), phosphorylase 'a' and 'b' ( $\mu$  moles of inorganic phosphate/mg protein/hr) in control, cathodal and anodal halves of the amphibian gastrocnemius muscle

|                   | Control<br>C1 + C2<br>2 | KH             | % change<br>over<br>control | AH             | % change<br>over<br>control | % change<br>KH over AH |
|-------------------|-------------------------|----------------|-----------------------------|----------------|-----------------------------|------------------------|
| Protein           | 23.0 $\pm$ 2.0          | 24.2 $\pm$ 1.1 | +5.4<br>N.S.                | 17.3 $\pm$ 3.1 | -21.8<br>$p < 0.05$         | +40.1<br>$p < 0.01$    |
| Glycogen          | 1.4 $\pm$ 0.2           | 1.5 $\pm$ 0.3  | +2.0<br>N.S.                | 1.2 $\pm$ 0.4  | -1.6<br>N.S.                | +20.9<br>N.S.          |
| Phosphorylase 'a' | 31.8 $\pm$ 2.3          | 33.4 $\pm$ 2.5 | +4.7<br>N.S.                | 21.5 $\pm$ 3.4 | -21.6<br>$p < 0.05$         | +43.2<br>$p < 0.05$    |
| Phosphorylase 'b' | 77.1 $\pm$ 12.0         | 79.2 $\pm$ 9.0 | +2.6<br>N.S.                | 51.2 $\pm$ 4.6 | -35.5<br>$p < 0.01$         | +11.4<br>$p < 0.05$    |

Values are mean  $\pm$  S.D. of 6 observations; + or - indicates increase or decrease respectively.

may be due to higher calcium content and calcium precipitable proteins in this muscle half<sup>17</sup>, and may not be due to electromigration of the enzyme as such since the pI of phosphorylase is 6.8 which is the same as that of the homogenate pH. It has been shown that high levels of AMP and low levels of ATP enhance the activity of phosphorylase 'a'<sup>16</sup>. The KH was shown to have high levels of AMP and low levels of ATP compared to the AH<sup>18</sup> and these factors may contribute to the high level of activity of this enzyme in the KH. The AH has high levels of neutral protease and its associated lysis<sup>11</sup> when compared to the KH. Such increased proteolytic activity may bring about a degradation of cellular proteins and enzymes which may result in a decreased phosphorylase activity.

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# PHYSIOLOGY OF YELLOW MOSAIC VIRUS IN GREEN GRAM, *PHASEOLUS AUREUS* ROXB. WITH REFERENCE TO ITS PREFERENCE BY *EMPOASCA KERRI* PRUTHI.

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It has been observed by many workers that virus infected leaves are preferred by aphids, thrips and leafhoppers, due to the accelerated conversion of the leaves to the stage most acceptable for insects, by virus invasion. However, the physiological basis of such a preference has not been clearly understood in many instances. A study of imbalance in the major metabolic processes in virus infected plants would help to understand precisely the mechanisms responsible for this preference. With a view to studying the impact of yellow mosaic virus disease on green gram on the infestation of the leafhopper, *Empoasca kerri* Pruthi., a non-vector of the virus, counts were made under field conditions on adult leafhopper population.

During the early hours of the day, population was assessed by suddenly trapping the leafhoppers in a plant with the help of a polyethylene bag with least disturbance. A total of three counts were made at an interval of five days. Altogether 126 plants in each of healthy and diseased plants were observed.

The leaf samples were taken from 27 days old plants and analysed for moisture, total nitrogen,<sup>1</sup> different forms of nitrogen<sup>2,3</sup>, phosphorus, calcium, magnesium, potassium<sup>4</sup>, free amino acid, sugar and organic acid content. The free amino acids of the leaf samples were extracted in 80% ethanol and estimated by uni-dimensional descending paper chromatography using *n*-butanol-acetic acid-water (4:1:5) as solvent system. The spots were identified by developing colour with 0.1% ninhydrin in acetone. The individual spots were eluted in alcohol-buffer mixture and quantitatively estimated by colorimetry. The soluble sugars were also estimated by paper chromatography using 80% hot alcohol for extraction and *n*-butanol-acetic acid-water (4:1:5) as solvent system<sup>5</sup>. The sugars were identified using benzidine in acetic acid and quantitatively estimated by eluting in water and developing colour with anthrone reagent<sup>6</sup>.

The leafhopper population was found to be consistently higher on diseased plants throughout the period of observation indicating their preference for the diseased plants (Table I). It has been reported that the virus-infected plants are the most favourable hosts for vectors like *Orosius albicinctus* Dist.<sup>7</sup> and non-vectors like *Empoasca kerri*<sup>8</sup> and *Amrasca devastans*<sup>9</sup>.

TABLE I  
Preference of leaf hoppers to yellow mosaic diseased and healthy green gram plants  
(Number of adults/plant—Means of 42 observations)

|                 | Age of plants in days |      |      |      |
|-----------------|-----------------------|------|------|------|
|                 | 22                    | 27   | 32   | Mean |
| Healthy         | 2.34                  | 2.38 | 2.90 | 2.54 |
| Diseased        | 4.30                  | 5.10 | 6.24 | 5.22 |
| S.E.            | 0.34                  | 0.24 | 0.32 | 0.06 |
| C.D. (P = 0.01) | 1.26                  | 0.88 | 1.22 | 0.22 |

Though total nitrogen was less in the diseased plants, different forms of nitrogen, viz., non-protein, nitrate, nitrite, ammoniacal and amide and total free amino acids were observed to be in higher quantities (Tables II and III). This is in accordance with the findings of Ramiah<sup>10</sup> and Regupathy and Jayaraj<sup>9</sup> in yellow vein mosaic diseased bhendi.

TABLE II  
Nitrogen make-up of healthy and diseased green gram (% on dry weight basis—Means of 3 observations)

|                      | Healthy | Diseased | % increase (+)<br>or decrease<br>(-) from<br>healthy |
|----------------------|---------|----------|--|
| Total Nitrogen       | 5.53    | 4.54     | - 7.9  |
| Nitrate Nitrogen     | 0.175   | 0.210    | +20.0  |
| Nitrite Nitrogen     | 0.175   | 0.210    | +20.0  |
| Ammoniacal Nitrogen  | 0.356   | 0.400    | +12.4  |
| Amide Nitrogen       | 0.175   | 0.280    | +60.0  |
| Non-protein Nitrogen | 0.280   | 0.380    | +35.7  |

TABLE III  
Free amino acid make-up of healthy and diseased green gram µg/1.0 g fresh tissue  
(Means of 3 observations)

|  | Healthy | Diseased | % increase (+)<br>or decrease<br>(-) from<br>healthy |
|--|---------|----------|--|
| Cystine                                  | ..      | 300      | ..   |
| Histidine and Lysine                     | 662     | 876      | +32.3  |
| Glycine                                  | 806     | 950      | +17.9  |
| Threonine                                | 1450    | 1826     | +25.9  |
| Alanine                                  | ..      | 426      | ..   |
| Tyrosine                                 | 1276    | 1350     | + 5.8  |
| Valine                                   | 350     | 350      | ..   |
| Leucines                                 | ..      | 300      | ..   |
| Unidentified (in terms of glutamic acid) | 550     | 950      | +72.7  |
| TOTAL                                    | 5094    | 7328     | +43.9  |

The higher nitrite nitrogen in the diseased leaves was due to the increased nitrate reductase activity<sup>10</sup> and the increase in the levels of ammoniacal and amide nitrogen may be due to the conversion of nitrogen into these forms to meet the requirements of virus for its own multiplication. The ammoniacal nitrogen content had a positive relationship with total amino acid content<sup>11</sup>. The diseased leaves differed from the healthy ones in their amino acid content quantitatively as well as qualitatively. Out of nine amino acids detected, only six were present in healthy leaves, viz., histidine (and lysine), glycine, threonine, tyrosine, valine and one unidentified ( $R_f$ —0.374). Infected leaves, in addition, had cystine, alanine and leucines. Except valine, all others were found to be on the increase in diseased leaves. Several records of enhanced amino acid metabolism in viroseed plants have been made<sup>9,12</sup>. Out of the six amino acids, listed by Mittler<sup>13</sup>, that strongly enhance the feeding of aphids, leucines were present only in diseased leaves. Though the enhancement of feeding by threonine, tyrosine and glycine is slight, their effect could be felt when the quantity is more. The chemotactic influence of amino acids on *E. kerri* may be similar to that reported in *E. fluvescens*<sup>14</sup>.

Total sugar content was less in diseased leaves (Table IV) with no qualitative difference between healthy and infected leaves. Fructose and sucrose were less in diseased leaves while glucose content was more. Vidhyasekaran and Kandasamy<sup>15</sup>

TABLE IV

*Soluble sugar make-up of healthy and diseased green gram  $\mu\text{g}$  1.0 g fresh tissue  
(Means of 3 observations)*

|          | Healthy | Diseased | % increase (+)<br>or decrease<br>(-) from<br>healthy |
|----------|---------|----------|--|
| Fructose | 4532    | 2000     | -55.9  |
| Glucose  | 6132    | 8132     | +32.6  |
| Sucrose  | 12932   | 10532    | -18.6  |
| Total    | 23596   | 20664    | -12.4  |

observed a decrease in total sugar contents in the mosaic infected green gram tissue. The decreased photosynthetic activity<sup>16</sup>, coupled with the increased respiratory rates<sup>12</sup>, generally observed in virus infected leaves should have led to decreased concentration of sugars. Though sugars have phagostimulatory role in inducing feeding of insects, Nuorteva<sup>17</sup> observed that leafhoppers avoided increased quantity of sugars and the same phenomenon has been reported earlier<sup>9</sup>.

There was no appreciable difference in moisture and phosphorus content between healthy and diseased leaves. While the reduction in magnesium

and potassium content was high due to virus infection, that of calcium was less (Table V). The

TABLE V

*Moisture and mineral make-up of healthy and diseased green gram (% on dry weight basis—Means of 3 observations)*

|            | Healthy | Diseased | % increase (+)<br>or decrease<br>(-) from<br>healthy |
|------------|---------|----------|--|
| Moisture   | 79.9    | 79.7     | -0.3   |
| Phosphorus | 0.29    | 0.30     | +3.4   |
| Calcium    | 1.76    | 1.69     | -4.0   |
| Magnesium  | 1.59    | 0.57     | -64.2  |
| Potassium  | 1.54    | 1.34     | -13.0  |

reduced calcium content of the diseased plant is in conformity with a similar report by Jeyarajan and Ramakrishnan<sup>18</sup> in chilli plants infected with potato virus Y, by Regupathy and Jayaraj in yellow vein mosaic diseased bhendi<sup>9</sup> and phyllody diseased sesamum plants<sup>7</sup>. As calcium is required for strengthening the cell wall of the plants, its decrease may induce the plants vulnerability to stylet and ovipositor penetration of jassids.

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## LETTERS TO THE EDITOR

### DIELECTRIC PROPERTIES OF SOME ESTERS AT 9214 MHz AND 1 MHz

WE have carried out experimental investigations on some esters at two frequencies, one, a microwave frequency of 9214 MHz and the other, a radio-frequency of 1 MHz. Using the standing-wave technique, dielectric absorption studies at the above-mentioned microwave frequency have been carried

radio frequency measurements, in general, agree fairly well. The observed values of radio frequency measurements are in close agreement with those of literature, wherever available<sup>1-5</sup>. The relaxation times increase for the higher members of a homologous group as expected. The calculated activation energies for dipole orientation  $E_\tau$ , and for viscous flow  $E_\eta$ , seen to be reasonable.

TABLE I

|                      | $\mu$ in<br>Debye units | Microwave $\tau$<br>in Picoseconds | RF $\mu$ in<br>Debye units | $E_\tau$ | $E_\eta$ |
|----------------------|-------------------------|------------------------------------|----------------------------|----------|----------|
| 1. Ethyl propionate  | 1.74                    | 4.2                                | 1.69                       | 1.93     | 2.95     |
| 2. Butyl acetate     | 1.65                    | 5.5                                | 1.89                       | 2.08     | 2.95     |
| 3. Amyl acetate      | 1.74                    | 7.4                                | 1.87                       | 2.26     | 2.95     |
| 4. Butyl butyrate    | 1.60                    | 6.8                                | 2.05                       | 2.21     | 2.95     |
| 5. Amyl butyrate     | 1.64                    | 8.7                                | 2.00                       | 2.36     | 2.95     |
| 6. Benzyl benzoate   | 2.44                    | 4.6                                | 2.49                       | 1.98     | 2.95     |
| 7. Dimethyl malonate | 2.36                    | 4.8                                | 2.42 (2.40)                | 2.00     | 2.95     |
| 8. Diethyl malonate  | 2.44                    | 5.6                                | 2.53 (2.57)                | 2.05     | 2.95     |

Values in parantheses are taken from literature.

out at room temperature  $25 \pm 1^\circ \text{C}$ , on eight esters in dilute solution in benzene. The dielectric constants at 1 MHz and refractive indices for the sodium D line, of dilute solutions of these compounds in the same solvent have also been determined.

The relaxation times  $\tau$  and dipole moment  $\mu$  have been evaluated from these measurements using the methods due to Gopal Krishna<sup>1</sup> and Guggenheim<sup>2</sup>. The free energies of activation have also been calculated using Eyring's<sup>3</sup> equations by evaluating the frequency factor at the temperature of measurements. The results of these measurements and calculations are given in Table I.

Dipole moments are accurate to about 3% and relaxation times, to 15-20%.

From Table I, it may be seen that the static dipole moments are a little higher than microwave dipole moments; such discrepancies are also reported in the literature. Dipole moments of homologous molecules like acetates, butyrates, malonates, etc., deduced either from microwave or

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### ULTRAVIOLET ABSORPTION SPECTRA OF ISOMERIC PYRIDINEALDEHYDES LONGEST WAVELENGTH $n-\pi\pi$ SYSTEM

ALTHOUGH the near UV absorption spectra of pyridine<sup>1,2</sup> and monosubstituted pyridines with ortho-para directing substituents like alkyl<sup>2</sup>, halogens<sup>3,4</sup> and amino<sup>5</sup> have been extensively studied,

there is very little work on the corresponding vapour absorption spectra of monosubstituted pyridines with meta-directing substituents. Vapour absorption spectra of cyano pyridines have been studied recently<sup>6</sup>. In present note, the vapour absorption spectra of isomeric pyridinealdehydes in the near ultraviolet region are reported.

From theoretical considerations<sup>7</sup>, it is concluded that meta directing substituents are expected to shift the non-bonding electron transition to the longer wavelength side. Mason<sup>8</sup> on the basis of the fact that para position shares maximum charge of the promoted non-bonding electrons, predicts maximum shift of  $n-\pi^*$  transition of pyridine in the 4-isomer. Del Bane and Jaffé<sup>9</sup>, on the other hand, suggest that the maximum shift of  $n-\pi^*$  transition is expected for the 2-isomer. The present experimental investigation on the spectra of pyridine aldehydes was undertaken to resolve this discrepancy.

TABLE I

Principal excited state fundamentals of pyridinealdehydes observed for the  $n-\pi^*$  system to anition

| Compound           | 0, 0 band<br>cm <sup>-1</sup> | $\nu_{C=O}$ cm <sup>-1</sup> | Fundamentals modes           |                          |                             |
|--------------------|-------------------------------|------------------------------|------------------------------|--------------------------|-----------------------------|
|                    |                               |                              | $\nu_{18a}$ cm <sup>-1</sup> | $\nu_1$ cm <sup>-1</sup> | $\nu_{13}$ cm <sup>-1</sup> |
| 2-pyridinealdehyde | 26398                         | 442<br>(551)*                | 763<br>(833)                 | 965<br>(996)             | 1191<br>(1215)              |
| 3-pyridinealdehyde | 26562                         | 421<br>(571)                 | 794<br>(833)                 | 990<br>(1026)            | 1164<br>(1217)              |
| 4-pyridinealdehyde | 26295                         | 456<br>(578)                 | 790<br>(807)                 | 935<br>(994)             | 1124<br>(1218)              |

\* Values in brackets are the corresponding ground state frequencies observed in the vibration spectra.

The longest wavelength system extending from 380–330 nm in all the three isomeric pyridinealdehydes consists of an extended system of sharp line-like bands distinctly separated from the diffuse system adjoining on the shorter wavelength side. That the sharp system corresponds to ( $n-\pi^*$ ) transition was established on the basis of the theoretical calculations referred to above, and, on the solvent effect studies. In 4-isomer, which belongs to  $C_{2v}$  symmetry, this corresponds to  $^1(B_2-A_1)$  transition and in 2 and 3-isomers with  $C_s$  symmetry, this corresponds to  $^1A''-^1A'$  transition. The transitions are symmetry allowed in all the three cases. All the bands observed in this transition, in case of 2-, 3- and 4-pyridinealdehydes have been analysed. The (0,0) band has been assigned in all cases on the basis of intensity variations, due to temperature effect. The bands at 26398, 26562 and 26295 cm<sup>-1</sup> are assigned to the (0,0) bands in 2-, 3- and 4-pyridinealdehydes respectively. Table I gives these as also the various ground and excited state frequencies observed in the system. Our results show that the shift of  $n-\pi^*$  transition is maximum

for the 4-isomer and this is in agreement with the prediction by Mason, and not of Jaffé *et al.*<sup>9</sup>, according to whom maximum shift is expected for the 2-isomer.

There is still another point of interest in the present series of compounds. In pyridinealdehydes two types of  $n-\pi^*$  transitions are expected namely the one arising from the aldehydic oxygen and the second arising from pyridine nitrogen. Comparison of the observed spectrum with those of other monosubstituted pyridines and excitation of the normal modes, corresponding to the ring vibrations, suggest that the observed spectrum corresponds to  $n-\pi^*$  transition of pyridine nitrogen and not due to the carbonyl group.

In an earlier note<sup>10</sup>, it was shown that the three isomers give only phosphorescence emission, arising from the  $^3(n-\pi)$  transition involving non-bonding electrons of the ring nitrogen. The ques-

tion of the location of the non-bonding electron singlet and triplet states from the aldehydic carbonyl oxygen therefore remains still undecided.

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# ETCH HILLOCKS ON SELENIUM

HARRISON AND SAGAR<sup>1</sup> produced pseudo-hexagonal pits on the {0001} planes and {10 $\bar{1}2$ } pyramid planes of melt grown selenium, using bromine dissolved in methanol, as the etchant. One to one correspondence was found on the match faces of {10 $\bar{1}2$ } planes. {10 $\bar{1}0$ } faces did not reveal any pit formation. Henrion and Eckart<sup>2</sup> carried out etch studies on vacuum-sublimed needles of selenium. Concentrated sulphuric acid at 150°C revealed flat etch patterns in the form of rounded rectangles. The present author has carried out etching studies on melt-grown single crystals of selenium using aqueous potassium hydroxide.

A freshly cleaved sample was kept in 60% KOH at 60°C for 20 minutes. The crystal was taken out and rinsed with distilled water, methanol and ether successively and then dried in hot air.

Electron microscopy revealed that the etch features were hillocks. In order to find whether these hillocks were at dislocation sites, the sample was cleaved and the match faces had the correspondence between the hillocks on the two faces. In order to confirm, that these hillocks were at dislocation sites, the surface of the crystal was etched and then indented. The crystal was then subjected to second etching. When observed under the microscope a characteristic gathering of hillocks around the indentation mark was found.

When the etch-studies were carried out for the (10 $\bar{1}0$ ) prism plane, the hillocks were at the dislocation sites on the prism plane. Basal plane did not give favourable results thus confirming that the hillocks were at the dislocation sites.

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## KINETICS OF Ag<sup>+</sup> CATALYSED OXIDATIVE DECARBOXYLATION OF SOME ORGANIC ACIDS BY Ce<sup>4+</sup> IN H<sub>2</sub>SO<sub>4</sub> MEDIUM

In the oxidation of various organic acids, Willard and Young<sup>1</sup> observed that formic and acetic acids were inactive towards ceric sulphate. However, many of these acids were oxidised when ceric nitrate<sup>2</sup> or

ceric perchlorate<sup>3</sup> was employed as an oxidant. Recently, it is reported that many of the aliphatic acids could be decarboxylated using a variety of oxidants<sup>4-6</sup>. In our earlier work<sup>7</sup>, it was shown that Ag<sup>+</sup> acts as a good catalyst in the oxidations involving ceric sulphate and hence, it was thought worthwhile to employ Ce<sup>4+</sup>-Ag<sup>+</sup> system in the oxidation of organic acids.

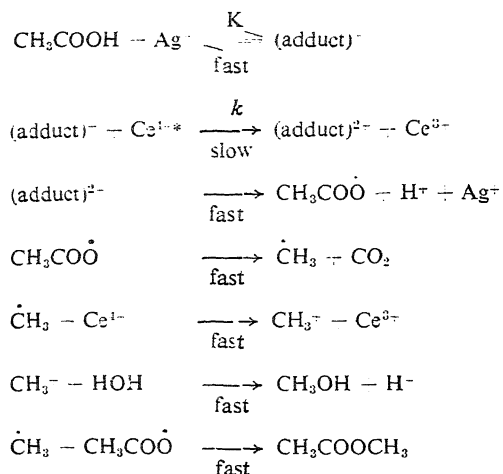
All the chemicals used were of reagent grade and the method of following the kinetics was the same as in our earlier paper<sup>7</sup>. Under conditions of [Ce<sup>4+</sup>]  $\ll$  [organic acid] in the presence of constant amount of [Ag<sup>+</sup>] the order of [Ce<sup>4+</sup>] was found to be unity. The pseudo-first order rate constants (*k'*) were calculated from the slope of the plot of log *a/a-x* vs. time. The order with respect to all the organic acids obtained from the log *k'* vs log [organic acid] plots was found to be fractional. Increase in [Ag<sup>+</sup>] increases the rate and the order of [Ag<sup>+</sup>] was also fractional in all the cases. [Ag<sup>+</sup>] was found to be unchanged at the end of the reaction as indicated by the constant thiocyanate titre value. Increase in [H<sup>+</sup>] from 0.1 M (*k'* = 7.9  $\times$  10<sup>-3</sup> min<sup>-1</sup>) to 0.7 M (*k'* = 21.9  $\times$  10<sup>-3</sup> min<sup>-1</sup>) at 52°C and at constant ionic strength ( $\mu$  = 1.9 M) shows that H<sup>+</sup> accelerates the rate. Increase in sulphate or bisulphate concentration decreases the rate. The reactions were studied in the temperature range 45-65° to evaluate the activation energy.

Ag<sup>+</sup> catalysis of Ce<sup>4+</sup> oxidations was first observed by Sinha<sup>8</sup> in the Ce<sup>4+</sup> - Ti<sup>3+</sup> reaction. The order of unity observed for [Ag<sup>+</sup>], [Ce (NO<sub>3</sub>)<sub>6</sub>]<sup>2-</sup> and [Ti<sup>3+</sup>] was explained with the aid of Ag<sup>2+</sup> assumed to have been formed from the reaction between Ag<sup>+</sup> and a complex species TiH[Ce (NO<sub>3</sub>)<sub>6</sub>]. Higginson *et al.*<sup>10</sup> also postulated an intermediate Ag<sup>2+</sup> in the Ti<sup>3+</sup> and Hg<sub>2</sub><sup>2+</sup> oxidations by Ce<sup>4+</sup>. Both these groups of workers assumed that the oxidation of Ti<sup>3+</sup> takes place by Ag<sup>2+</sup> in a subsequent step. One significant observation of the latter workers is that in the absence of a suitable substrate no reaction was possible between Ce<sup>4+</sup> and Ag<sup>+</sup>. The fractional order of organic acid indicates that it may be involved in the complex formation either with Ce<sup>4+</sup> or Ag<sup>+</sup>. Spectral studies in the present work indicated no complexation between Ce<sup>4+</sup> and organic acid in conformity with our earlier observations<sup>8</sup>. This, as well as the effect of [H<sup>+</sup>], [sulphate], and [bisulphate], which shows a similar trend reported earlier for other substrates, indicates that neutral Ce (SO<sub>4</sub>)<sub>2</sub> is the reactive species. Ag<sup>+</sup> is known to form colourless adducts with oxygen containing compounds with a lone pair of electrons on oxygen atom<sup>11</sup>. It is therefore not unreasonable to assume



the formation of an adduct between  $\text{Ag}^+$  and organic acid before oxidation by  $\text{Ce}^{4+}$  occurs in a slow step to yield  $\text{Ag}^+$ -substrate adduct. The formation of  $\text{Ag}^+$ -substrate adduct was confirmed by adding bipyridyl to the reaction system which gave brown coloured bipyridyl complex of  $\text{Ag}^+$  with its characteristic absorption maximum at 454 nm<sup>12</sup>.

If the adduct formation is taken as the first step, the reaction scheme for the  $\text{Ce}^{4+}$  -  $\text{CH}_3\text{COOH}$  reaction (taken as a typical example) in the presence of  $\text{Ag}^+$  could be written as follows :



\*  $\text{Ce}(\text{SO}_4)_2$  is written as  $\text{Ce}^{4+}$  for simplicity.

From the above mechanism the rate equation comes out to be

$$-\frac{2.303 d \log [\text{Ce}^{4+}]}{dt} = k' = \frac{Kk [\text{Ag}^+] [\text{CH}_3\text{COOH}]}{1 + K [\text{CH}_3\text{COOH}] + K [\text{Ag}^+]} \quad (1)$$

where  $k'$  is the observed pseudo first order rate constant obtained from the plot of  $\log a/a-x$  vs time,  $k$  is the bimolecular rate constant for the slow step and  $K$  the formation constant of the adduct. Equation (1) accounts for the first order dependence of rate on  $[\text{Ce}^{4+}]$  and fractional order dependence on  $[\text{Ag}^+]$  and [organic acid] obtained experimentally.

Taking the reciprocal of the equation (1) we get

$$\frac{1}{k'} = \frac{1}{[\text{CH}_3\text{COOH}]} \left[ \frac{1}{Kk [\text{Ag}^+]} - \frac{1}{k} \right] + \frac{1}{k [\text{Ag}^+]} \quad (2)$$

From equation (2) it is clear that the plots of  $1/k'$  vs  $1/[\text{organic acid}]$  at constant  $[\text{Ag}^+]$  should be linear. Such plots were obtained in the present work for all the acids studied. From the intercept and slope the bimolecular rate constant for the slow

step ( $k$ ) and formation constant for the adduct ( $K$ ) were evaluated (Table I).

TABLE I

| Name of the acid       | $k \times 10^3$<br>l mol <sup>-1</sup> sec <sup>-1</sup> | $K$ l mol <sup>-1</sup> | $\Delta E^\ddagger$ Kcal<br>mol <sup>-1</sup> |
|------------------------|--|-------------------------|---|
| Acetic acid            | 5.6  | 1.09                    | 22.9  |
| Propionic acid         | 21.0   | 1.20                    | 22.0  |
| Iso-butyric acid       | 29.7   | 9.27                    | 18.3  |
| Pivalic acid           | 45.0   | 33.89                   | 13.1  |
| <i>n</i> -Butyric acid | 22.2   | 2.24                    | 17.7  |
| <i>n</i> -Valeric acid | 23.1   | 3.50                    | 17.2  |
| Iso-valeric acid       | 23.0   | 4.50                    | 13.7  |

Due to inductive effect of the methyl groups, its substitution in  $\text{CH}_3\text{COOH}$  reduces the strength of the corresponding acids. The inductive effect of the alkyl groups increases in the order:  $\text{CH}_3 < \text{CH}_3\text{CH}_2 < (\text{CH}_3)_2\text{CH} < (\text{CH}_3)_3\text{C}$ <sup>13</sup>. The increase in electron density on hydroxyl oxygen favours the formation of  $\text{Ag}^+$ -carboxylic acid adduct. This is clearly indicated from the formation constants calculated from kinetic studies in the present work (Table I). For the same reason the bimolecular rate constants ( $k$ ) for the slow step should also show a similar trend. That this is so, is clear from Table I. The acidity constants data<sup>13</sup> indicate that *n*-butyric, *n*-valeric and iso-valeric acids are weaker than acetic acid and hence, should have higher  $K$  value. The  $K$  and  $k$  values follow this trend (Table I) and confirm indirectly the formation of  $\text{Ag}^+$ -acid adducts.

The plot of  $\Delta H^\ddagger$  vs  $\Delta S^\ddagger$  is linear with a slope equal to 350 which is the isokinetic temperature. The isokinetic temperature is greater than the temperature range used in the present study (318–338° K) indicating that the reactions are enthalpy controlled, as is evident from Table I.

The authors wish to express their gratitude to Professor N. V. Subba Rao, Head of the Department of Chemistry, Osmanina University, for his keen interest in this work.

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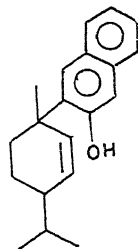
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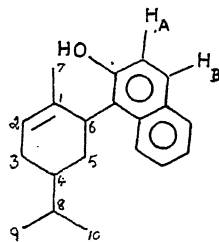
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# REVISED STRUCTURE FOR THE ADDUCT OF $\alpha$ -PHELLANDRENE WITH $\beta$ -NAPHTHOL

INCORPORATION of  $\beta$ -naphthol to  $\alpha$ -phellandrene gave an adduct  $C_{26}H_{24}O$ , m. p. 139–140° for which Salfeld<sup>1,2</sup> assigned structure (I). We now propose structure (II) for this derivative based on its spec-



I



II

tral properties: ir (nujol): 3400 ( $-\text{OH}$ ), 1630

( $\text{>C=C<}$ ): 1380, 1375, 1165 ( $-\text{CH}<\begin{smallmatrix} \text{CH}_3 \\ \text{CH}_3 \end{smallmatrix}$ );

$850\text{ cm}^{-1}$  ( $\begin{smallmatrix} \text{R}_1 \\ \text{R}_2 \end{smallmatrix}\text{C}=\text{C}\begin{smallmatrix} \text{R}_3 \\ \text{H} \end{smallmatrix}$ ): nmr ( $\text{CCl}_4$ ) (60 MHz);

$\delta$  7.7 (multiplet, 4 H; aromatic protons); 7.60, 7.47, 7.07, 6.94 (AB quartet, J 8 Hz; 2 H; aromatic protons  $\text{H}_A$  and  $\text{H}_B$ ); 6.1 (singlet, 1 H;  $-\text{OH}$ ); 5.5 (broad singlet  $\text{W}_{\frac{1}{2}}$  8 Hz; 1 H;  $\text{C}_2\text{H}$ ); 4.1 (broadened doublet,  $\text{J}_{6,5ax}$  11 Hz,  $\text{J}_{6,5eq}$  small; 1 H;  $\text{C}_5\text{H}$ ); 1.7 (doublet, J 1 Hz; 3 H;

$\text{C}_7-\text{CH}_3$ ); 0.8 [obscured pair of doublets; J 7 Hz; 6 H; ( $-\text{CH}<\begin{smallmatrix} \text{CH}_3 \\ \text{CH}_3 \end{smallmatrix}$ )].

Further details will be published elsewhere.

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## OCCURRENCE OF *KECKIA ANNULATA* GLOCKER, IN THE BAGH BEDS OF NARMADA VALLEY

OCCURRENCE of *Keckia annulata* Glocker a Cenomanian Trace Fossil from Germany, in the Nimar Sandstone (Fig. 1) is of interest because of the information it yields about the ethological conditions during the deposition of the Bagh Beds.



FIG. 1. *Keckia annulata* Glocker, showing feeding burrows along bedding plane and holes approaching the bedding plane,  $\times 1$ .

*Keckia annulata* Glocker, 1841

**Material.**—Several tubes on slab No. Ph 1:74: **Dimensions:** length 10.5 — 5.5 cm; width 1.2 cm; wall thickness 0.25 cm.

**Remarks.**—Circular to broadly elliptical cross section, distinct and uniformly thick tube wall without any constrictions or rugosities and habit of crossing over from one bedding plane to another and branching there, help us in identifying our material with Glocker's species<sup>6</sup>.

The present feeding burrows come from the Astarte Bed at Phata, only a few metres away from where we have collected *Palaeodictyon* sp. Its habit of crossing over from one bedding plane to another and branching there indicates that *Keckia* at times accepts food from suspension, though in general, it is a sediment feeder, and thus it falls in Seilacher's<sup>7</sup> ethological group of Fodichnia.

Therefore, *Keckia* belonging to the zoophycus facies, associated with *Palaeodictyon* of the nereites facies<sup>7</sup>, is of interest, as it indicates its occurrence in a zone, partly of zoophycus facies and partly of nereites facies. Such a position is ascribed by Seilacher<sup>7</sup> to *Palaeodictyon* from the Mississippian and Pennsylvanian turbidite series of Ouachita Mountains.

Majority of the other trace fossils reported from the shaly intercalations near the top of the Nimar Sandstone in Amba Dongar area<sup>1,2,5</sup> indicate cruziana facies. Occurrence of *Palaeodictyon* and *Keckia annulata* in a similar horizon at Phata indicates deepening of the basin during its deposition.

*Astarte flexicostata* Chip. & Bad., *A. sinuicostata* Chip. & Bad., *Protocardium pondicherricense* d'Orb., *Cordium phataensis* Chip. & Bad. and *Turritella chiklensis* Chip. & Bad. occur in Astarte Bed<sup>3,4</sup> along with the present Trace Fossil: they are of a type burrowing through sediment for their food and belong to the *Macoma* community occurring from the tidal zone down to 60–80 fathoms, or even more, but indicating estuarine conditions<sup>8</sup>.

Trace fossils are not good indicators of geological age: even then it is of interest to note that *Keckia annulata* occurs in the Cenomanian of Germany which is also the age of the Astarte Bed of Phata.

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### THE STRATIGRAPHIC DISTRIBUTION OF *MEYERIPOLLIS* BAKSI AND VENKATACHALA IN THE TERTIARY OF ASSAM

The genus *Meyeripollis* was instituted by Baksi and Venkatachala<sup>3</sup> to circumscribe "gemmate, trisyncolpate microfossil grains" observed in the Tertiary sediments of Assam. This type of plant microfossils, first reported from India by Meyer<sup>8</sup>, has been reported by a number of workers from Assam prior to Baksi and Venkatachala and since. It is worthwhile to note that Sah and Dutta<sup>9</sup> did not report this type from the same region.

In recent studies on the Tertiary subgroups of Garo Hills, the present author and his colleagues have recorded this taxon in the cores and well-cuttings from Boldamgiri, Inoligiri and Darik Members of Kherapara, and Rewak Formations in fairly good concentrations (*ONGC unpub. rep.*, 1973). Inoligiri and Darik Members of Kherapara Formation have been equated by Chakraborty and Baksi<sup>4</sup> and Chakraborty *et al.*<sup>7</sup> with the Barail Formation of Khasi Hills and Upper Assam on the basis of lithological, petrological and microfloral contents while Boldamgiri and Rewak Formations are considered younger and older respectively to these. Chakraborty *et al.*<sup>7</sup> have stated *Meyeripollis* to be an index fossil, having been "recorded only from the time-equivalent sediments of the Barail Group" in Assam and Bengal basin. The occurrence in fairly good concentrations of the taxon in the Boldamgiri and Rewak Formations in the subgroups of Garo Hills evidently indicates that *Meyeripollis* is not restricted only in the Barail or its equivalent formations as contended by Baksi<sup>1,2</sup>, Chakraborty and Baksi<sup>4</sup> and Chakraborty *et al.*<sup>7</sup>. As such the taxon is not reliable as an "index fossil".

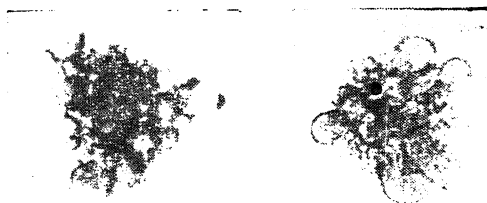


FIG. 1. Two species of *Meyeripollis* recorded from the subsurface of Garo Hills, Assam.

*Meyeripollis* has been recorded so far by this author invariably in association with pollen grains referable to *Barringtonia*. *Cocos* and other plants of littoral/coastal areas. Incidentally, *Barringtonia* type of fossil pollen is not an "index fossil" for Barail Formation or for Oligocene age as contended by Chakraborty *et al.*<sup>7</sup> having been recorded from older formations and horizons by Banerjee *et al.*<sup>8</sup>, Venkatachala and Kar<sup>10</sup> and other workers.

In Assam region, *Meyeripollis* has been observed to be almost restricted in the Barail Formation in Upper Assam while in Central and Lower Assam it is present in both Pre- and Post-Barail sediments, while shallow marine to brackish-water conditions during Paleogene sedimentation in Assam is established. Banerjee and Misra<sup>6</sup> have shown the prevalence of similar conditions during Early Neogene sedimentation in Garo Hills, Surma Valley and Tripura in contrast to the contemporary continental depositional condition in Upper Assam. From this and the general microfloral association with *Meyeripollis*, it seems more probable that the occurrence of the taxon is more controlled by the ecological conditions during sedimentation rather than by the precise geological age or other characters of the Formation from which it is recorded.

The affinities of *Meyeripollis* has remained doubtful till now. The external morphology of the grains seem to be more akin to spores than to pollen grains. It would be appropriate to leave the question open, at present, to further studies.

The views expressed are those of the author only.

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## NOTE ON THE STROMATOLITES IN THE PAKHAI SERIES

The occurrence of stromatolite structures in the dolostones of Pakhal series exposed north of Daryapur (Lat. 16° 32' 30" ; Long. 79° 35' 30") in Kakinada District of Andhra Pradesh has been reported for the first time. The area consists of sandstones, pebbly sandstones and dolostones, and their order of succession has been established in the field as shown in Table I.

TABLE I  
*Lithologic succession*

- e. Ferruginous sandstone and ferruginous shales with laterite capings;
- d. Sandy dolostones, dolostones with development of stromatolites.
- e. Pebbly sandstones and glauconitic sandstones.
- b. Dolostones with cherts.
- a. Basal conglomerates and sandstones.

The Pakhal sediments comprise of two formations of carbonate sediments, lower dolostones and upper dolostones. On the basis of colour, these are further subdivided into two types as grey and pink dolostones. Grey dolostones are succeeded by pink dolostones and show typical chopmarked weathering. On the basis of chemical composition of the carbonate sediments as given in Table II, these are

TABLE II  
*Chemical composition (partial) of the chip samples of dolostones*

| Specimen No. | Locality                  | Type of dolostones | CaO   | MgO   | Ca Mg ratio |
|--------------|---------------------------|--------------------|-------|-------|-------------|
| 51           | 2 km north of Daryapur    | Grey dolostones    | 28.84 | 18.34 | 1.57        |
| 52           | "                         | Pink dolostones    | 26.74 | 18.04 | 1.48        |
| 57           | 3 km NE of Daryapur       | Grey dolostone     | 27.44 | 18.65 | 1.47        |
| 68           | 2 km east of Daryapur     | Pink dolostone     | 27.86 | 17.34 | 1.6         |
| 79           | 4 km SW of Ramakrishnapur | Grey dolostone     | 26.74 | 17.03 | 1.56        |
| 80           | 1½ km NE of Kesanpalli    | Pink dolostone     | 27.44 | 18.64 | 1.47        |
| 33           | 1½ km SW of Sitampet      | Pink dislostone    | 28.14 | 20.46 | 1.37        |

classified as dolostones to magnesian dolostones after Chillinger (1957).

A stromatolitic bed is exposed in the upper dolostones, 2 km. east of Daryapur and runs up to 2 km. in NNE-SSW direction. It varies in thickness from less than 1 metre to 2 metres. It occurs as nodule-like (Serebrykov *et al.*, 1974) form being embedded in fine calcareous mud.

In the stromatolitic bed two distinctive bioherms are identified. The dominant form is *collenia symmetrica*, Fenton and Fenton (Fig. 1) which occurs throughout the bed, and is accompanied by *Collenia columnaris* Fenton and Fenton, (Fig. 2).

(i) *Collenia symmetrica* Fenton and Fenton :

They start from a point on the substratum and grow upwards by addition of convex upward laminae, increasing in area as the colony develops. Being depressed subcircular hemispheroids in shape, and, diameter varying between 1 and 5 cms., they have a height ranging from 2 to 4 cm.

*Collenia symmetrica* is found in the lower part of the Siyeli Formation of Piegan Group, Belt Series of U.S.A., (Fenton and Fenton, 1937). The Baikal Formation of the Burzyan Series (Lower Riphean) of the Southern Urals also contains *Collenia symmetrica*.

(ii) *Collenia columnaris* Fenton and Fenton :

The species have columnar axis which is inclined at 70° to 80° to the bedding plane. The columns are generally separated by clastic carbonate material and lime-mud, which widen slightly upwards. In the transverse section they are nearly circular to oval. The growth is due to the addition of convex upward laminae that do not increase greatly the surface area. These species have a diameter of 1 to 3 cms. at the base.

*Collenia columnaris* occurs in the Hell Roaring member of the Altyn Limestone, Ravalli group, Belt series of northwestern United States (Fenton and Fenton 1931, 1937), and Burzyan series (Lower Riphean) and Arzyan Series (Middle Riphean) of Southern Urals of U.S.S.R. (Keller *et al.*, 1960).

The stromatolites occurring in the Pakhal dolostones may be correlated with lower to Middle Riphean formations (Older than 1260 my) of U.S.S.R. More detailed investigations on the nature of stromatolites, their occurrence within the dolostones and the probable chemical equilibrium conditions are in progress.

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#### INTRACELLULAR ORGANIZATION OF LEECH BODY WALL MUSCLE FIBRES: HISTO- ENZYMOLOGICAL PROFILE

IN an earlier report<sup>1</sup> it was described that the muscle fibres of leech body wall show histological/histochemical differentiation into a peripheral (cortex) and a central (core) region. Positive staining was observed for lipids, glycogen, succinic dehydrogenase (SDH) and cytochrome oxidase in the sarcoplasmic core of the muscle fibres, whereas the cortex was apparently devoid of these metabolites and enzymes<sup>1</sup>. The cortex, however, showed strong staining for protein and PAS reaction (after saliva treatment). It was suggested thereby<sup>1</sup> that the cortical region had an essentially 'contractile' function, while the sarcoplasmic core was the site of active metabolic functioning-yielding energy for fibre contraction. The present investigation describes the contractile status of the cortical 'myofilaments' by the application of m-ATPase reaction.

Locally collected leeches (*Hirudinaria granulosa*) were anaesthetised with light ether and stretched to normal length. Small transverse pieces from the anterior, middle, and posterior regions of the body were cut, frozen in cryostat and sectioned at 10  $\mu$ . Alternatively, the body pieces were fixed in Bouin's fluid, embedded in paraffin and 8  $\mu$  sections were processed for histological staining by Mallory's triple and iron-haematoxylin techniques. SDH and m-ATPase activities were demonstrated in unfixed fresh frozen sections by the Nitro-BT technique of Nachlas *et al.* and calcium method of Padykula and Herman as described by Pearce<sup>2</sup>.

The assignment of contractile activity to the fibre cortex<sup>1</sup> was based on the observation of numerous fine filaments running across the cortical space (Fig. 1). However, this was only a gross histological observation, and inconclusive towards establishing the contractile nature of the cortical 'myofilaments'. In the present investigation, differentiation of the individual muscle fibres into cortical and core regions was apparent both in the histological (Fig. 1) as well as histochemical (Figs. 2, 3) preparations. Histochemically, though SDH activity was almost exclusively confined to the sarcoplasmic core

NOTE ON THE STROMATOLITES IN THE PAKHAL SERIES

By P. H. REDDY

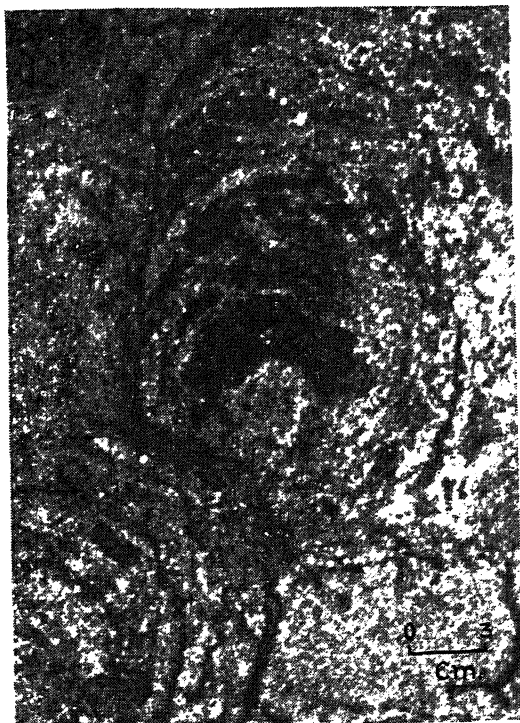


FIG. 1. *Collenia symmetrica* Fenton and Fenton.



FIG. 2. *Collenia columnaris* Fenton and Fenton.

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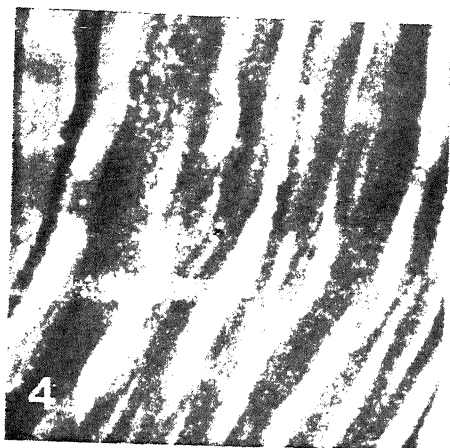
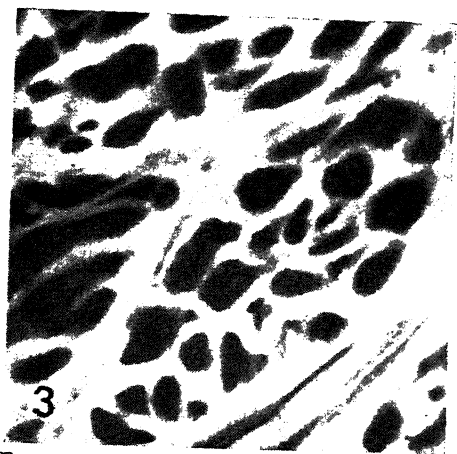
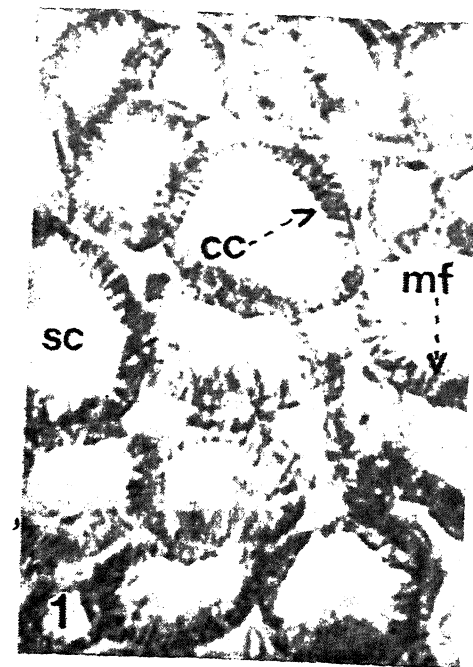
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region (Fig. 3). m-ATPase activity and an obvious cortical localization (Fig. 2). The fibres of the circular, oblique, dorso-ventral and longitudinal muscles of the body wall had an almost identical histological/histochemical profile.

Contractile proteins can be visualized by a technique for demonstration of activated ATPase activity of the muscle fibres. It is well recognized as for the specific histochemical detection of myosin-associated ATPase activity. Thus, demon-



FIGS. 1-4. Fig. 1. Histological preparation of leech body wall muscle fibres. Cross section. Mallory's Triple stain,  $\times 400$ . mf, myofilaments; c, contractile cortex; sc, sarcoplasmic core. Fig. 2. Myosin-adenosine triphosphatase activity in the muscle fibres. Note exclusive localization of the enzyme activity in the cortex region (peripheral),  $\times 400$ . Inset,  $\times 35$ . Figs. 3 and 4. Succinic dehydrogenase activity in the cross and longitudinal sections of leech body wall muscle fibres. Note exclusive staining of the central core region,  $\times 100$ .

$\text{Ca}^{2+}$ -activated ATPase activity of the muscle fibres has been established to be associated with their

stratification of m-ATPase activity exclusively in the cortical region of the leech body wall muscle. Cl-



suggests the presence of 'myosin like' contractile protein. The m-ATPase activity of the cortex may perhaps eventually be ascribed to the cortical 'myofilaments' revealed in the histological preparation. Even if the cortical m-ATPase activity is not really located in the cortical filaments observed histologically, it suggests the presence of the contractile protein (myosin) exclusively in the cortex. The present observation, therefore, establishes the contractile status, exclusively to the cortex of the muscle fibres, in substantiation of our earlier histological observation).

We thank Mr. E. A. Daniels, for photomicrographic assistance.

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January 22, 1975.

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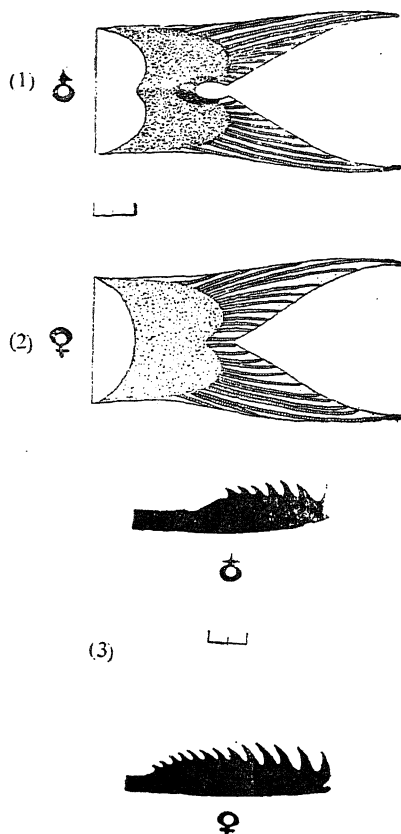
### SEXUAL DIMORPHISM IN *PSEUDEUTROPIUS ATHERENOIDES* (BLOCH)

For the present study several hundred specimens of *Pseudeutropius atherenoides* in 50 mm-85 mm size range, were collected from the lakes and rivers of Gorakhpur and Faizabad during the months of June, July and August 1974.

The authors have not come across any account of sexual dimorphism in *Pseudeutropius atherenoides* but careful examination reveals that they do display sexual dimorphism in having a thickening at the base of the caudal fin ending in a crescent-like structure at the level of the fork in males (Fig. 1). In females the thickening does not form a crescent and it never reaches the fork (Fig. 2). Such thickening at the level of the fork in caudal fin has been reported in *Mystus (M.) vittatus* by Swarup and Swaroop<sup>1</sup> but it is spear-shaped.

Also, the males have well developed genital papilla (5-7 mm) and 7 to 9 denticulations on the pectoral spine (Fig. 3). In females the genital papilla is feebly developed and the pectoral spine

denticulations are more in number (13) (Fig. 3). Day<sup>2</sup> has described about ten pectoral spine denticulations in this fish and Srivastava<sup>3</sup> has given thirteen as the number of these denticulations but none of them has linked this character with sex.



FIGS. 1-3. Fig. 1. Showing thickening at the base of the caudal fin in male *P. atherenoides*. Note the crescent-like area of the thickening. Scale 2 mm. Fig. 2. Showing thickening at the base of the caudal fin in female *P. atherenoides*. Fig. 3. Showing denticulations on the pectoral spine of male and female *P. atherenoides*. Scale 2 mm.

Department of Zoology,  
University of Gorakhpur,  
Gorakhpur, January 25, 1975.

KRISHNA SWARUP,  
ANAND SWAROOP.

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# TUBO-UTERINE JUNCTION IN TWO SPECIES OF INDIAN BATS

LITERATURE reveals that details of the tubo-uterine junction are known in very few mammals, and even among these, there are considerable differences in the anatomy and histology of this region of the female genitalia. Hafez and Black<sup>1</sup> recognized five types of tubo-uterine junction in the mammals they studied. Among bats, the tubo-uterine junction has been described in two species. *Tadarida brasiliensis cynocephala* (Stephens)<sup>2</sup>, where the Fallopian tubes enter at the apex of each cornu, slightly towards the median aspect, via an oviductal papilla, and *Glossophaga soricina* (Rasweiler IV<sup>2</sup>) where the uterus is simplex and the oviducts enter the uterus cranially (fundically). In the latter species, the extramural junctura has a well developed tunica muscularis and a densely fibrous lamina propria. These layers are reduced in the intramural junctura and are finally lost as the intramural junctura penetrates the lamina basalis of the endometrium. Since so little is known about the tubo-uterine junction in bats, and since the species under study present new situations, it was felt that a study of this part of the genital anatomy would be of interest. The species studied here are *Rousettus leschenaulti*

(Megachiroptera—Pteropidae) and *Hipposideros fulvus fulvus* (Microchiroptera—Hipposideridae).

In *Rousettus leschenaulti* (Fig. 1) the Fallopian tube is short and takes a simple curve laterally after passing across the ventral aspect of the respective ovarian bursa, and penetrates the uterine cornu a little behind the cranial end of the uterus on the mesometrial side. Examination of serial sections of this region reveals that the Fallopian tube takes a sharp bend after it meets the uterus and passes through the uterine muscle layers obliquely for some distance before it enters the endometrium of the uterus. Throughout its course, within the muscle layers of the uterus, it is provided with its own muscle layers which remain distinct from the muscles of the uterus almost up to the region when it directly opens into the cranial end of the uterine lumen by a dilated opening. The muscle layers of the Fallopian tube are absent, only for a very short segment near its distal end where its connective tissue merges with the endometrium of the uterus. The epithelial folds of the Fallopian tube are present throughout its length within the uterine wall.

In *Hipposideros fulvus fulvus* the Fallopian tube on each side arises from the median margin near the caudal end of the ovarian bursa. It takes a

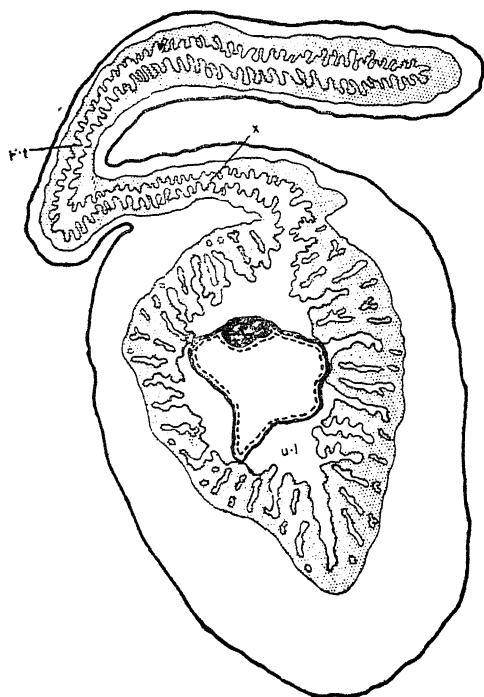


FIG. 1. Schematic representation (reconstructed from serial sections) of the tubo-uterine junction in *Rousettus leschenaulti*.

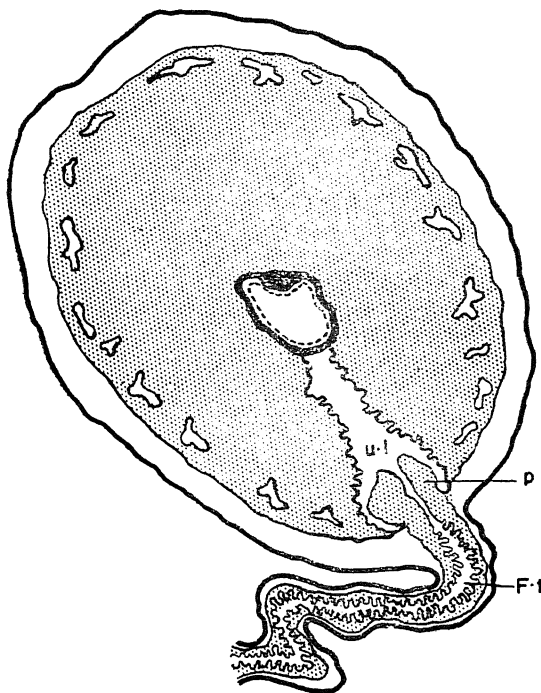


FIG. 2. Schematic representation (reconstructed from serial sections) of the tubo-uterine junction in *Hipposideros fulvus fulvus*.

turning round the middle of the ovarian capsule and bend suddenly on the side of the ovarian capsule, piercing the uterine wall perpendicularly, and opens on a distinct papilla on the mesometrial aspect of the uterine lumen, a little caudal to the anterior end of the uterus. The Fallopian tube does not have the folding of its epithelium after it pierces the muscularis of the uterus, and the muscularis layer of the Fallopian tube becomes imperceptibly merged with the muscle layers of the uterus. Hence, the papilla is mostly made up of connective tissue of the endometrium. Figure 2 illustrates the anatomy of the tubo-uterine junction in this species.

From the foregoing it would appear that even within the same order of mammals the anatomical relationship between the Fallopian tube and the uterus varies considerably among the different species.

I am grateful to Prof. Dr. A. Gopalakrishna, Director, Institute of Science, Nagpur, for guidance and encouragement during the progress of this work.

Department of Zoology,  
Institute of Science,  
Nagpur, March 23, 1975.

K. B. KARIM.

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#### A REPORT ON THE OCCURRENCE OF EGG SAC IN *HETERODERA MOTH* (NEMATODA: TYLENCHIDA)

*Heterodera mothi*, a cyst nematode was described from the roots of *Cyperus rotundus* by Khan and Hussain<sup>1</sup> from the University campus, Aligarh. Yaquooob and Khan<sup>2</sup> found maximum number of viable cysts of this nematode during November, the cysts number decreased gradually from December onwards till June. The same species has recently been recorded by Minton *et al.*<sup>3</sup>, from the roots of yellow nutsedge, *Cyperus esculentus* from Georgia, U.S.A. However, neither Khan and Hussain nor the subsequent authors reported about the formation of egg sacs in the adult females of *H. mothi*. The presence or absence of egg sacs in cyst nematodes is of taxonomic significance.

In August 1974, a large number of white females of *H. mothi* were isolated from the roots of *Cyperus rotundus* in Aligarh. It was interesting to note that a majority of these females were having egg sacs attached to their hinder part of the body. The egg sacs were hard in nature and nearly as big or bigger than the females themselves. When

the females were removed from the roots, the egg sacs usually got detached inspite of every possible care, during removal. In nature, perhaps the same phenomenon occurs and the egg sacs remain attached to the host roots even when the females die. On many occasions dry egg sacs containing empty egg shells, attached to the host roots were collected. The females would have obviously dropped the egg sacs in the soil upon death.

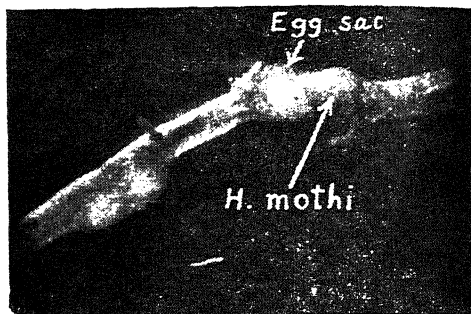


FIG. 1. Showing *Heterodera mothi* along with its egg sac on the root of *Cyperus rotundus*.

The white females of *H. mothi* were seen on *C. rotundus* only from July to October. Afterwards they, transformed into cysts whose number was maximum during November and December. After December, the number of cysts gradually decreased. It was also observed that the eggs which were retained within the cysts did not hatch without stimulation (e.g., root diffusates), while those in the egg sacs hatched in large numbers even in tap-water without any stimulation.

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Aligarh, India, April 23, 1975.

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#### ON A NEW SPECIES OF *ANOPILOPHRYA* STEIN (PROTOZOA: CILIATA: ASTOMATIDA) FROM AN INDIAN OLIGOCHETA

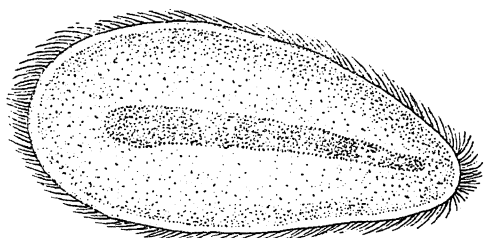
Not much work has been done in India on Ciliate parasites occurring in the digestive tract of Oligochaeta. Some of the important references on the subject are Ghosh (1918, 1921), Ghosh, Haldar and Chakraborty (1970), Biswas and Mukherjee (1974).

We have been engaged, for some time, on researches on one of the highly controversial groups

of Ciliates, viz. astomates, commonly found in the alimentary canal and coelome of Oligochaeta. During the course of these investigations we came across a new species of the genus *Anoplophrya* Stein in the alimentary tract of *Perionyx excavatus* Perrier, from Barrackpore, West Bengal.

It is described in the present paper. The description is based partly on the observation on living animals and partly on stained examples. The material was fixed in Schaudin's fluid and stained in Heidenhain iron haematoxyline and counterstained with eosine.

#### DESCRIPTION



50/ $\mu$

FIG. 1. *Anoplophrya anilii* Mukherjee and Chakrabarti.

*Anoplophrya anilii*, n. sp.

Body elongately oval, anterior end broadly rounded, gradually narrower towards post pole, posterior end rounded, thickly and uniformly ciliate; Contractile vacuoles three observed in living forms. Macronucleus more or less band shape extending two-third of the body; micronucleus small, spherical placed by the side of the macronucleus. Clear distinction in the endoplasmic zone is well pronounced.

**Measurement:** Body—L  $85 \mu \times B 48 \mu$ ; Macro-nucleus— $58 \mu$ .

**Types.** Holotype on slide; Paratype—2 specimens on slides will be deposited in the National collection of Z.S.I.

**Host:** *Perionyx excavatus* Perrier.

**Type locality of host:** Barackpore, 24-Parganas, West Bengal, India; Date 16th April 1971. Coll. A. Chakrabarti.

**Remarks:** Among all the species of *Anoplophrya* described so far *A. anilii* n. sp. resembles *A. lumbrici* (Shrank) slightly in shape. *A. lumbrici* is easily distinguishable from the new species under report, in having the shape of body somewhat pointed at anterior end and distinctly curved longitudinally, i.e., dorsal side convex and ventral side concave in shape.

The name *Anoplophrya anilii* is proposed for this new species after the name of a renowned protozoologist, Dr. Anil Mandal.

The authors are grateful to Dr. K. K. Tiwari, Dr. J. K. Sen, Shri K. N. Nair and Dr. S. Khera, Deputy Director-in-Charge of the Zoological Survey of India, for constant encouragement and guidances during the study and preparing manuscript.

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Calcutta 700016, January 27, 1975.

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#### HISTOPATHOLOGY OF *DASYCHIRA MENDOSA* Hb. (LEPIDOPTERA : LYMANTRIIDAE) INFECTED WITH NUCLEAR POLYHEDROSIS VIRUS

AN epizootic of nuclear polyhedrosis of *Dasychira mendosa* Hb. was reported for the first time by Rabindra and Subramaniam (1974). With a view to understand the host-pathogen relationship, histopathological investigation was taken up using virus infected fourth and fifth instar caterpillars of *Dasychira mendosa* Hb.

Infected caterpillars in different stages of disease were fixed in alcoholic Bouin's fixative (Dubosque Brazil), washed in 70% ethyl alcohol, dehydrated in ethanol-butanol series and embedded in paraffin, according to standard procedures. Sections 4–6  $\mu$  were stained by a modified azan staining technique after Hamm (1966). The pathological changes in the various tissues as observed in the light microscope are presented below.

The principal tissues, found to be susceptible, were fat body, tracheal matrix, hypodermis, nerve cells (Fig. 1) and blood cells. According to Aizawa (1963) and Smith (1967) fat body, hypodermis, tracheal matrix and blood cells are the chief sites of multiplication of the virus. However, in the present instance, infection of other tissues such as muscles, connective tissues surrounding the midgut and testicular epithelium were also found to contain polyhedra in the nuclei. Infection of these tissues has been reported by Benz (1963) in

*Malacosoma diploca* and Hamm (1968) in *Spodoptera frugiperda*.

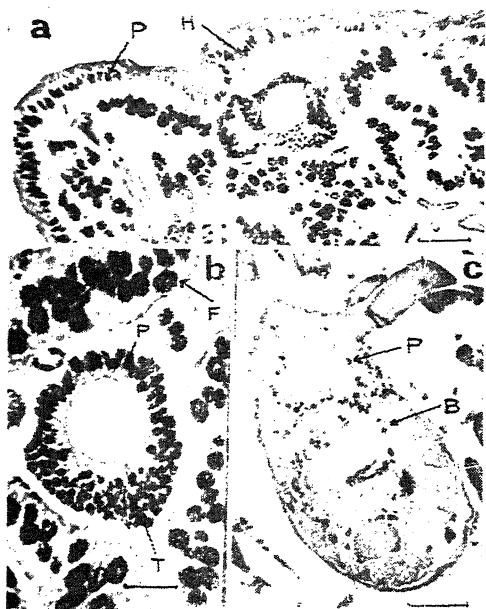


FIG. 1. Cross section of nuclear polyhedrosis virus-infected *Dasychira mendosa* showing polyhedra (P) in hypertrophied nuclei of *a*, hypodermis (H). Line = 0.14 mm; *b*, Tracheal matrix (T) and fat body (F). Line = 0.07 mm; *c*, nerve cells of brains (B). Line = 0.106 mm.

Early signs of infection could be observed in fat body, hypodermis and trachea 48 hours after infection. Infection of other tissues like blood cells, nerve sheath and gonads could be observed only 72 hours after inoculation. In the case of fat body it was noticed that polyhedra in certain nuclei were larger than those in the adjacent nuclei. These nuclei were found to rupture earlier releasing the polyhedra. Silk glands, foregut and malpighian tubules were not found to be affected by the virus.

Dept. of Entomology, R. JEBAMONI RABINDRA,  
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Research Institute,  
Coimbatore 641003, February 5, 1975.

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## PHYTOPHTHORA PARASITICA—A NEW RECORD FROM SOUTH INDIA ON TOMATO

A TOMATO fruit rot, hitherto undescribed from South India, was found for the first time in July 1973 at the Main Research Station, Bangalore. The same rot was also observed in August 1974 on the tomato variety 'Pusa Ruby' and some other selections resistant to the root-knot nematode. The disease was noticed only after the onset of rains when the weather conditions were warm and wet. The rot was observed to affect about 30% of the tomato fruits under field conditions.

The rot is usually confined to the fruits in any stage of development. More often the fruit rot is marked by one or more fairly broad, irregular zones of alternating shades of brown and grayish brown, forming a typical buckeye effect (Fig. 1). The rot is hard at first and the fruits rapidly decay and breakdown in a semi-rot later. A white, fluffy mold appears on the surface of the rot during rainy weather in advanced stage of fruit decay.



FIG. 1. Buckeye effect of tomato rot caused by *Phytophthora parasitica*.

Repeated isolation from the tissues of the tomato fruit affected with the rot invariably yielded *Phytophthora parasitica* Dastur usually in pure cultures. Inoculations of tomato fruits, detached from the plant and undetached, and of all stages with pure cultures of the fungus, invariably resulted in reproduction of the rot. Repeated reisolations made from artificially infected fruit yielded again *P. parasitica*.

*P. parasitica* has been reported to cause damping-off of tomato seedlings by Singh and Srivastava (1953)<sup>1</sup> from Uttar Pradesh. This organism has also been reported by Rao (1966)<sup>2</sup> from Maharashtra State to cause soft rot of tomato in market and storage. Likewise Sharma (1974)<sup>3</sup> reported that this fungus causes buckeye rot of tomato in Himachal Pradesh. This is the first report of this organism on tomato from South India.

The culture of *P. parasitica* has been deposited in Type Culture Collection of the Department of Plant Pathology, University of Agricultural Sciences, Bangalore, under Accession No. 119.

The author wishes to acknowledge Dr. H. C. Govindu, Senior Professor and Head, Department of Plant Pathology, University of Agricultural Sciences, Bangalore, for his keen interest and encouragement during the course of this investigation.

Department of Plant Pathology,  
University of Agricultural Sciences,  
Bangalore, February 8, 1975.

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### MINIPLANT-TUBES FOR STUDIES ON VIRUS TRANSMISSION WITH LEAF HOPPER VECTORS

IN plant virus transmission studies, the viruliferous insects are enclosed for inoculation along with test seedlings, inside the cages of lantern glasses or cellulose butyrate tubes pressed into the soil in pots. Such cages occupy large area and also get soiled easily. Further, many insects under study escape while transferring. A simple method using transparent plastic tubes (3" × 1") with cup-like push-in lids eliminates all these disadvantages.

The lids serve as supporting cups for growing plants when filled with soil or vermiculite. One or two pinholes, made in the base, form the passage for the entry of water and nutrients from outside. Sprouted seeds are placed on the soil medium in the cups which are arranged on a layer of soil-manure mixture (3 : 1), supplemented with fertilizers. When the trays are watered, the soluble nutrients of the mixture in the tray are carried into the cup through pinholes.

The bottom of the tube is cut and a fine mesh muslin cloth is stuck in place by briefly dipping the end in acetone, ether or chloroform and gently pressing it against the cloth. The tube, when placed in position over the cup, forms a complete independent enclosure for the seedling and the insect (Fig. 1). Such plant tubes are simple, easy to handle and require very limited space. They can be arranged in racks and kept either in green houses or growth chambers under artificial illumination. Very young seedlings, soon after sprouting, can be inoculated. It is easy to transfer the insects from plant to plant, as the cups are easily detached along

with the seedlings, while retaining the insect in the tubes.

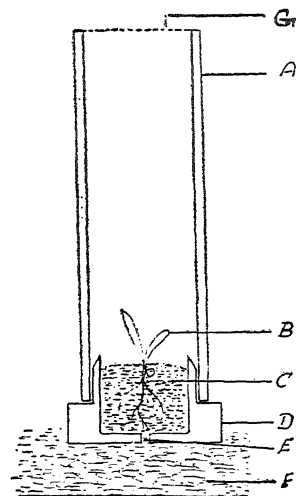


FIG. 1. Diagram of miniplant-tube. A, tube; B, Seedling; C, Soil + Sand; D, Lid; E, Pinhole; F, Soil + Manure; G, Muslin.

The author has used this method, with success, for growing and inoculating wheat, sorghum, finger millet, foxtail millet, pearl millet with a virus transmitted by *Cicadulina bipunctella bipunctella* and *C. chinai*. The uninoculated ragi plants remained green and normal but dwarf for two months in the plant tubes when kept either in green house or under artificial illumination.

The author wishes to thank the Indian Council of Agricultural Research, New Delhi and the University of Agricultural Sciences, Bangalore, for giving the financial assistance and facilities respectively. He also thanks Mr. T. M. Mustak Ali, Millets Scheme, University of Agricultural Sciences, for making the drawing and Dr. H. C. Govindu for his encouragement in this study.

Virologist, A.I.C.R.P. (Millets), H. R. REDDY.  
Main Research Station,  
University of Agricultural  
Sciences, Bangalore,  
January 30, 1975.

### A NEW PHYTOPHTHORA LEAF BLIGHT AND DAMPING OFF DISEASE OF PASSION FRUIT FROM INDIA

DURING a disease survey of local flora, one of us (B. A. U.) observed an outbreak of leaf blight of passion fruit (*Passiflora edulis* Sims.) at Horticultural Experiment Station, Chethalli, Coorg, in two successive monsoon seasons (1972-74). Damping

off symptoms were also noticed on the seedlings in the nursery. The disease incidence was sporadic initially but became serious with prolonged wet weather resulting into severe defoliation in grown-up plants and death of seedlings.

In nature, the disease appears with the onset of rains and the symptoms first appear on lowermost leaves. Subsequently, upper leaves, flowers and young fruits are also attacked. Discoloured to dull green areas appear initially and become more distinct as the colour of the affected tissues change to light tan (Fig. 1). The infected flowers and fruits are shed. Severe defoliation is noticed during favourable weather. On the seedling, irregular water-soaked areas develop at the collar region resulting into damping off.

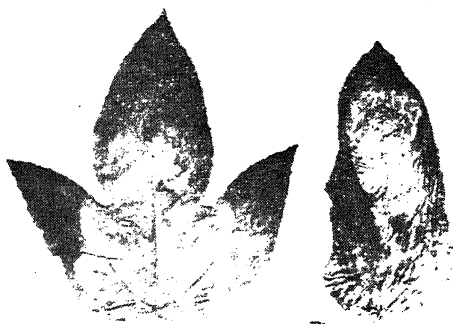


FIG. 1. Leaves of *Passiflora edulis* showing typical symptoms of leaf blight under natural conditions.

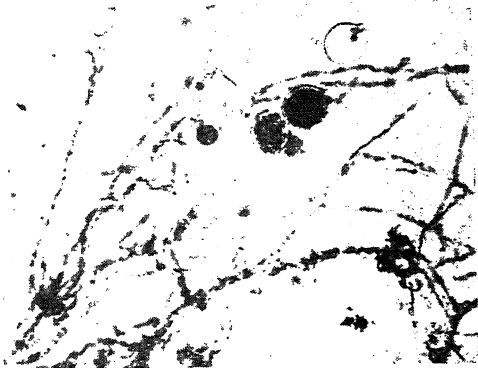


FIG. 2. Photomicrographs of passion fruit *Phytophthora* showing papillate and empty sporangia on oat meal agar.

*Phytophthora nicotianae* Br. deH. was repeatedly isolated from the diseased tissue (Fig. 2). It proved highly pathogenic on artificial inoculation producing characteristic symptoms in 72 hours. Purple

(Local), Purple (Kenya) and Yellow varieties were found susceptible. It also attacked the green fruits of Bell pepper and Tomato.

Earlier, *Phytophthora cinnamoni* Rands has been reported to cause root rot of passion fruit from New Zealand<sup>1</sup>, whereas *P. nicotianae* Br. deH. and *P. cinnamoni* have been responsible for root rot from Western Australia<sup>2</sup> and wilt caused only by *P. nicotianae* from S. Africa<sup>3</sup> and Sarawak<sup>4</sup>. None of these reports has established the association of *P. nicotianae* with leaf blight and damping off symptoms. This is, therefore, the first record of *P. nicotianae* van Breda-de Haan inciting leaf blight and damping off of passion fruit from India.

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#### DEVELOPMENT AND DIVERSITY OF STOMATA IN *MICROCOCOA MERCURIALIS* BENTH.

DUE to the great paucity of data on stomatogenesis in Euphorbiaceae, a fresh survey of stomatal structure and ontogeny was taken up for assessing the range of diversity in stomatal pattern and also to explore the implications of such data in systematic studies of Euphorbiaceae known for their extraordinary diversity in the vegetative, floral, anatomical, embryological and cytological characters.

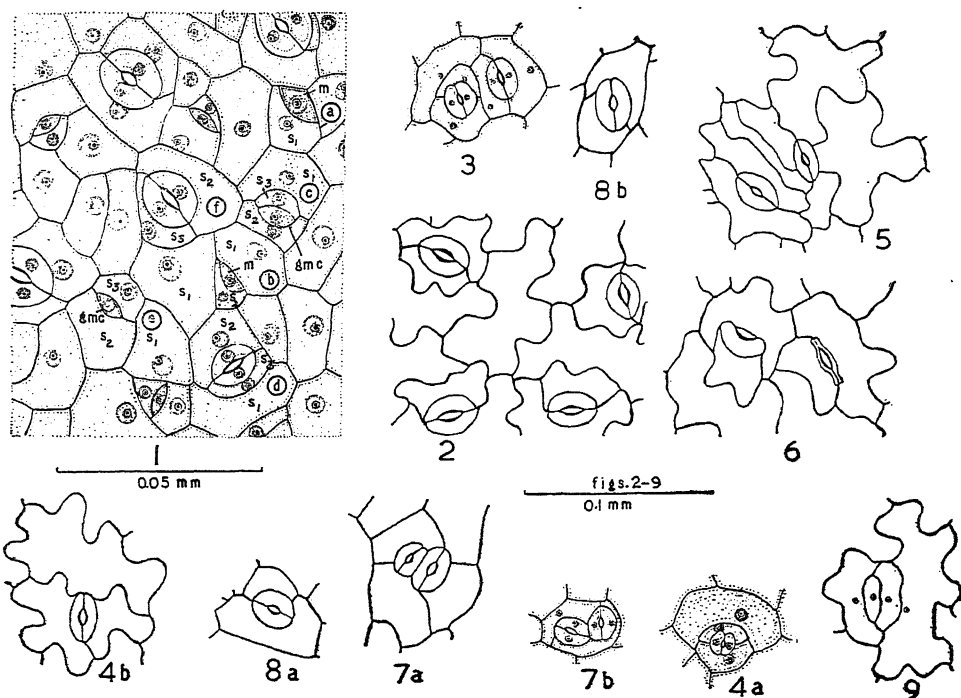
During the course of such an investigation, on several members of the family (about 30 species), interesting patterns of stomatal types were encountered in *Micrococca mercurialis*, of the tribe Acalypheae. According to the earlier workers<sup>1,2</sup> paracytic, anomocytic and anisocytic types of stomata occur in Euphorbiaceae. Besides these three types, a fourth type namely diacytic or caryophyllaceous type of stomata has also been observed in *Micrococca mercurialis*, a feature recorded for the first time in Euphorbiaceae.

In *Micrococca mercurialis*, the leaves are amphistomatic. The principal type of stomata is

of the paracytic type (92%), with occasional occurrence of the anomocytic (5.25%) and anisocytic (2.65%) types and rarely of the diacytic type (0.1%). The stomata are present on both abaxial and adaxial surfaces more or less in equal numbers and the frequency increases from base towards the apex. The stomata are diffusely oriented, mostly confined to and bordering the intercostal cells. According to Metcalfe and Chalk<sup>1</sup>, the stomata are usually confined to the abaxial surface only, except in the tribe *Crotonaeae*. But in the present study, the leaves are amphistomatic not only in *Micrococca mercurialis* of *Acalyphaeae*, but also in several genera of the various tribes studied.

The ontogeny of foliar stomata in *Micrococca mercurialis* is mesogenous. In young leaves, the protodermal cells are polygonal, richly cytoplasmic, straight-walled and uninucleate. The protodermal cell undergoes an unequal division and cuts off a smaller cell towards a corner with a curved wall which

constitutes the stomatal initial or meristemoid *m*, while the larger one behaves as the first subsidiary cell *s*<sub>1</sub>. The cell *m* is distinct by its shape, size, dense cytoplasm, position and presence of a large nucleus (Fig. 1 *a*). It cuts off a second subsidiary cell *s*<sub>2</sub> on the opposite side by a slightly curved wall which intersects the *s*<sub>1</sub> at both the polar ends of the *m* (Fig. 1 *b*). Subsequently the *m* delimits a third subsidiary cell *s*<sub>3</sub> opposite to *s*<sub>2</sub> and towards *s*<sub>1</sub>, it intersects *s*<sub>2</sub> at one or both the polar ends (Fig. 1 *c*). At this stage the *m* appears to be flanked by *s*<sub>1</sub> and *s*<sub>3</sub> on one side and *s*<sub>2</sub> on the other. It gradually assumes a lenticular shape having deeply stained cytoplasm and a prominent nucleus and behaves as a guard cell mother cell *gmc* (Fig. 1 *c*). This undergoes a median division parallel to the previous ones and forms the two guard cells which eventually assume a characteristic bean-shaped structure with a central pore (Fig. 1 *d*). Therefore the stomata are typically paracytic<sup>3,4,5</sup>



FIGS. 1-9. Fig. 1. Stomatogenesis in a young leaf peel (*a-d*: dolabrate; *e-f*: trilabrate paracytic types). Fig. 2. Mature stomata (abaxial surface). Fig. 3. Diacytic and paracytic colleagues. Fig. 4. (*a*) Diacytic stoma; (*b*) Anisocytic stoma. Fig. 5. Paracytic and anomocytic colleagues. Fig. 6. Stoma with a single guard cell and stoma with both guard cells degenerated. Fig. 7 (*a*) and (*b*). Juxtaposed and obliquely placed contiguous stomata, respectively. Fig. 8 (*a*) and (*b*). Transitional stages between paracytic and diacytic types. Fig. 9. Stoma with arrested development at *gmc* stage.

(*gmc* = guard cell mother cell; *m* = Meristemoid; *s*<sub>1</sub>, *s*<sub>2</sub> and *s*<sub>3</sub> = First, second and third subsidiary cells, respectively.)



and their ontogeny conforms to the mesogenous (Pant, 1965)<sup>6</sup> or mesogenous-parallelcytic type (Payne, 1970)<sup>7</sup> of dolabrate origin (Bir Bahadur *et al.*, 1970)<sup>5</sup>. Mesogenous paracytic stomata of trilabrate origin (Fig. 1 *e, f*) are also met with where the stomata are monocyclic (intermediate between helicocytic and allelocytic types as reported for *Ipomoea hederacea*)<sup>7</sup>.

Occasionally at the division of the *gmc*, the spindle orients in such a way (perpendicular to the previous ones instead of parallel) resulting in the formation of diacytic stomata of mesogenous diallelocytic type<sup>7</sup> in the place of the usual paracytic stomata (Figs. 3, 4 *a*). Sometimes in the trilabrate type of origin, at *gmc* formation, the *m* touches *s*<sub>1</sub> at one polar end and *s*<sub>2</sub> at the other and consequently the subsidiaries produced are gradually smaller, thus resulting in stomata of mesogenous anisocytic type.

All the four major stomatal patterns occurring in dicotyledons are present even in a single leaf peel as reported earlier for *Phylla nodiflora* (Pant and Kidwai, 1964)<sup>8</sup>. There is considerable variation in the number of encircling cells among the paracytic stomata: 25% are monocyclic, 14% are dicyclic, 59% are intermediate between mono- and dicyclic, while 2% are incompletely tricyclic (with 5 subsidiaries).

In addition to the normal stomatal pattern, there are several abnormalities like: (*a*) stomata with single guard cell (Fig. 6), (*b*) stomata with both guard cells degenerated (Fig. 6), (*c*) contiguous stomata of two types (Patel and Shah, 1971)<sup>9</sup>: (1) contiguous along their lateral sides, *i.e.*, juxtaposed with their pores placed parallelly (Fig. 7 *a*) and (2) contiguous at their poles with their pores placed obliquely (Fig. 7 *b*); (*d*) several transitional stages between paracytic and diacytic types (Figs. 8 *a, b*), (*e*) stomata with three-fourths portion enclosed by one cell and the remaining by another<sup>8</sup> (Fig. 8 *a*) and (*f*) stomata with arrested development at the *gmc* stage (Fig. 9) which serves as an indelible clue for stomatogenesis in mature leaves (Pant, 1965)<sup>10</sup>.

The remarkable diversity in the stomatal pattern may be due to: (*i*) the way of intersection of the meristemoid with the subsidiaries and (*ii*) the orientation of the spindle during formation of guard cells.

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### SONCHUS YELLOW VEIN VIRUS—A NEW VIRUS DISEASE OF SOWTHISTLE

DURING the summer of 1974, many plants of sowthistle, *Sonchus oleraceus* L., were showing bright yellow vein symptoms suspected to be due to a virus (Fig. 1).

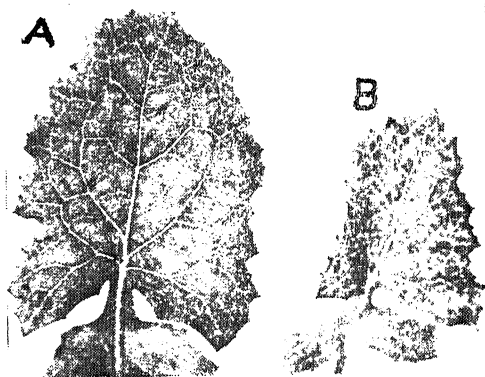


FIG. 1. Leaves of *Sonchus oleraceus* L. A. Healthy; B. Diseased showing yellow vein symptoms.

Attempts to transmit the virus by conventional method of sap inoculation were unsuccessful from sowthistle to sowthistle and lettuce. The virus was successfully transmitted to sowthistle by using young nymphs of aphids, *Dactynotus sonchii* Joelfrey. Non-viruliferous aphids were starved for 2 hr and given acquisition feeding period of 5, 10, 15, 30, 60 and 120 min on the diseased plant and later transferred on to the 20-day old test seedlings at the rate of 5 insects per seedling. They were allowed to feed for 24 hr and then transferred to fresh set of test plants at every 24 hr interval for 7 days. Symptoms of yellow veins appeared between 20–25 days after inoculation feeding on four out of eight plants where the aphids, receiving 10 min of acquisition feeding, were allowed to feed for the first 24 hr.

These aphids, however, failed to transmit further from second day onwards, thereby indicating that the virus is stylet borne. There is no report so far, of a virus producing yellow vein symptoms on *Sonchus* except the *Sowthistle yellow vein* virus reported by Duffus (1963), which differs from the virus under study, by having a long latent period in the vector, *Hyperomyzus lactucae*. Hence the authors consider this as a new virus of *Sonchus oleraceus* not reported so far.

The authors wish to thank Dr. H. C. Govindu, for providing necessary facilities. Thanks are due to Dr. Kanakaraj David, No. 1, 6th Street, B. V. Nagar, Madras 600020, for having identified the aphid.

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## ON ACTINASTRUM FROM JAMMU

A CONSISTENT AND CONSOLIDATED study on the Phytoplanktonic elements of some Kashmir lakes has recently been attempted by Kant and Kachroo<sup>1,2</sup>. In Jammu region, the area is still unexplored from phycological study point of view, except for a few reports by Kant<sup>3,4</sup>. A project has been undertaken to account for the algal and phytoplanktonic components of Jammu waters.

Jammu region represents a subtropical climate. The average atmospheric temperature and the rainfall for the months June and July, 1973 has been recorded as 28–32° C and 214.0 mm. respectively.

The author has collected many genera and species of algae during the course of his studies. But, of great interest, is *Actinastrum hantzschii* Lagerheim. The genus is being reported from Gadigarh Nallah in Jammu. The genus having a single species has also been reported from some States in India and from Ceylon<sup>5</sup>.

*Actinastrum hantzschii* Lagerheim<sup>6</sup>

(G. Lagerheim, 1882, p 70, pl 3, f 25–26; Smith<sup>7</sup>, G.M. 1920, p 164, pl 43, f 6–7; Philipose M.T. 1967, p 217, f 125 a–c). Fig. 1.

*Actinastrum hantzschii* Lage. is a colonial algae found in fresh waters. Colonies are generally of 4 or 8 cells radiating from a common centre. The colonies are 25–30  $\mu$  in diameter. The cells in a colony lie at an angle of 45°. The individual cells are cylindrical, with rounded ends, a smooth wall, a centric nucleus, parietal chloroplast and a pyrenoid.

The individual cells are 9.5–13.5  $\mu$  long and 2–3  $\mu$  broad.

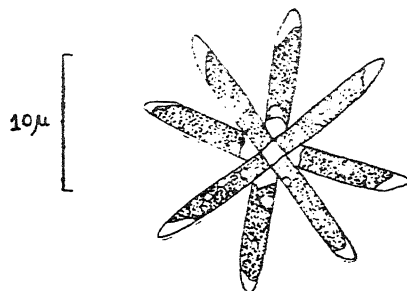


FIG. 1. A colony of *Actinastrum hantzschii* Lage.

*Habitat*.—pH fluctuates from 7.3 to 8.7; surface water temperature 25–30° C; a lot of emergent vegetation and Zooplanktons; June–July 1973.

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## INFLUENCE OF OXYTETRACYCLINE AND SULPHANILAMIDE ON THE AMINO ACID CONTENT IN THE MELONFLY, *DACUS CUCURBITAE* COQ.

THE adverse effects of oxytetracycline and sulphaniilamide on the development and reproduction of the melonfly, *Dacus cucurbitae* Coq. were reported by Sankaranarayanan and Jayaraj<sup>1</sup>. While investigating into the mechanisms of sterility-inducing action of these chemicals, Chinna- rajan *et al.*<sup>2</sup> showed the destruction of symbiotic micro-organisms in the mycetocytes of the midgut region. In the present study, the effect of these chemicals on the amino acid metabolism of the insect in the pupal and adult stages has been observed.

Two-days old larvae of the fruitfly were fed with pumpkin fruit pieces (*Cucurbita moschata* Duchs) soaked in 0.2% solutions of oxytetracycline and sulphaniilamide for 3 hr until they completed the larval development. The free amino acids of

newly formed pupae and female flies were estimated by two-dimensional paper chromatography<sup>6</sup>. The solvent systems, *n*-butanol-acetic acid-water (4 : 1 : 5) and phenol-water (85 : 15) were used, the colour developing reagent being ninhydrin 0.1%. The spots were eluted in alcohol-buffer mixture and read in a Spectronic-20, and the contents expressed as mg/g fresh weight (Table I).

Of the ten essential amino acids, required for normal growth in insects, like *Drosophila melanogaster* and *Tribolium confusum*<sup>4</sup> and *Hylemyia antiqua*<sup>5</sup>, and also for reproduction in *D. melanogaster* and *Aedes aegypti*<sup>6,7</sup>, four amino acids, viz., valine, leucine, isoleucine and threonine were found to occur in greater quantities in the pupa and adults of untreated insects (Table I). In addition, some

TABLE I  
*Effect of oxytetracycline and sulphanilamide (0.2%) on free amino acid contents in Dacus cucurbitae Coq.*

| Amino acids                     | Contents in pupa (mg/g) |                  |                 | Contents in female fly (mg/g) |                  |                 |
|---------------------------------|-------------------------|------------------|-----------------|-------------------------------|------------------|-----------------|
|                                 | Check                   | Oxytetra-cycline | Sulpha-nilamide | Check                         | Oxytetra-cycline | Sulpha-nilamide |
| Alanine                         | 10.66                   | 6.23             | 8.02            | 9.12                          | ..               | 1.94            |
| Glutamic acid                   | 10.36                   | 5.54             | 8.09            | 4.77                          | 1.14             | 2.50            |
| Glycine and or Serine           | 2.30                    | 2.12             | 1.00            | 0.17                          | 0.17             | 1.23            |
| Glutamine                       | 1.72                    | 0.49             | 0.58            | 2.54                          | 2.14             | 3.49            |
| Valine                          | 13.66                   | 4.69             | 7.21            | 2.40                          | 0.48             | 0.48            |
| Leucine and or Isoleucine       | 5.36                    | 0.13             | 0.20            | 2.43                          | 1.87             | 2.43            |
| Threonine                       | 2.67                    | 1.81             | 1.73            | 2.05                          | 1.14             | 1.36            |
| Aspartic acid                   | ..                      | ..               | ..              | 4.77                          | 1.14             | 2.50            |
| Tyrosine                        | ..                      | ..               | ..              | 1.21                          | ..               | ..              |
| No. of unidentified amino acids | 2                       | ..               | ..              | ..                            | ..               | ..              |
| Total content                   | 46.73                   | 21.01            | 26.83           | 27.54                         | 8.86             | 15.39           |
| % decrease from check           | ..                      | 55.04            | 42.58           | ..                            | 67.83            | 47.75           |

There were no qualitative differences in the amino acids between the normal and treated insects in the pupal stage except for the absence of two unidentified amino acids in the treated insects which were found in the normal ones. But the content was considerably reduced due to treatment with oxytetracycline (55%) and sulphanilamide (42.6%). The essential amino acids like glycine, valine, leucine, isoleucine and threonine were present in far lesser quantities in the treated pupae. Adult flies, on treatment with oxytetracycline and sulphanilamide, contained only 8.9 and 14.4 mg of amino acids respectively per gram of body weight of flies, while the quantity was as high as 27.5 mg in untreated insects. Tyrosine and alanine were not present in flies treated with oxytetracycline, while tyrosine alone was absent in sulphanilamide treated insects.

of the non-essential amino acids were needed to supplement essential ones for normal growth, development or egg production such as alanine, glycine, serine and tyrosine in *Agria affinis*<sup>8</sup>, and hydroxy proline, proline and serine in *A. aegypti*<sup>7</sup>. The absence of non-essential amino acids, viz., alanine, glutamic acid, aspartic acid, glutamine and tyrosine or presence only in smaller quantities in the treated insects now observed may be attributed for the poor growth and reproduction of fruitfly<sup>1</sup>. The number of amino acids has been reported by Pant and Kapoor<sup>9</sup> to have been reduced in the aposymbiotic pupae of *Lasioderma serricorne* F. compared to normal pupae.

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#### A SIMPLE TECHNIQUE TO IDENTIFY SCENT IN RICE AND INHERITANCE PATTERN OF SCENT

THERE are certain scented varieties of rice which on cooking give out an aroma. In these varieties aroma is also emanated from leaves. High milling returns and good cooking quality are associated with

$F_2$  populations of two crosses involving two strongly scented varieties *Randhuni* and *Kalabhat* (AC. 609), with *Hoyoku* a high yielding non-scented *japonica* cultivar during kharif 1974. Twenty-five days after transplanting, the leaves were analysed for scent on individual plant basis and the results were later confirmed by cooking the grain.

In both the cross combinations  $F_1$  was scented and  $F_2$  population segregated into 27 scented and 37 non-scented indicating that scent was controlled by the simultaneous actions of three dominant complementary genes (Table I).

Inheritance of scent has been reported to be monogenic dominant or recessive. A digenic segregation of 9:7, 15:1, 13:3 and trigenic ratio of 27:37 were also reported by various workers<sup>3</sup>. These workers studied scent by breaking rice grains under the teeth or by boiling the grain. Ramiah<sup>4</sup> pointed out varying ratios of scented to non scented were obtained owing to the difficulties in scoring method.

The technique adopted in the study offers a rapid method of identification of scent at early growth stages of plants. Leaf analysis should help to make effective selection from early generations in the field.

TABLE I  
Nature of segregation for scent in  $F_2$  population

| Cross                                 |      | Non-scented | Scented | Total | Ratio | $\chi^2$ | p value between |
|---------------------------------------|------|-------------|---------|-------|-------|----------|-----------------|
| Hoyoku $\times$<br>Randhuni           | Obs. | 201         | 159     | 360   |       |          |                 |
|                                       | Exp. | 208.13      | 151.87  | 360   | 37:27 | 0.24     | 0.50-0.30       |
| Hoyoku $\times$<br>AC. 609 (Kalabhat) | Obs. | 231         | 141     | 372   |       |          |                 |
|                                       | Exp. | 215.06      | 156.94  | 372   | 37:27 | 2.8      | 0.10-0.05       |

scented rice<sup>1</sup>. Some of the scented rice varieties are known for their tolerance to pests<sup>2</sup>. Hence breeding for high yielding scented rice varieties is an important problem.

Scent is a highly heritable character as some of the lines derived from *T. 412* (scented)  $\times$  *IR. 20* (non-scented) and the recently released high yielding 'Improved Sabarmati' show strong scent.

A simple technique is developed to identify scent from the leaf analysis. At tillering phase of rice, two to three leaves are excised from individual plants, cut into 1 cm long bits and kept in corked vials. The vials were warmed to 40°-45° C for 5 minutes to liberate volatile compounds. The aroma is then noted by smelling the contents of the vials. By this technique large population of plants can be analysed for scent without having to wait until harvest time and cooking the rice.

In the present investigation, inheritance pattern of scent by the above method was studied in  $F_1$  and

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# **PATTERN OF INHERITANCE OF BACTERIAL LEAF STREAK RESISTANCE IN RICE**

THERE is no available information on the inheritance pattern of bacterial leaf streak disease of rice (*Oryza sativa* L.) incited by *Xanthomonas translucens* f. sp. *oryzicola*. Studies on the inheritance of this disease were, therefore, initiated at the Central Rice Research Institute, Cuttack, during Rabi 1973. BJ-1 was used as the resistant parent and TKM-6 and IR-8 were the susceptible parents. Crosses involving these three parents were made during Rabi 1973 and the  $F_1$ ,  $F_2$  and  $F_3$  generations along with the parents were tested during the subsequent three seasons. The  $F_1$  plants were raised in earthen pots while  $F_2$  and  $F_3$  populations along with the parents were grown in the field for testing. Forty-five day old seedlings were spray inoculated using a bacterial cell suspension ( $ca 10^8$  cells/ml) obtained from a 48 hr old culture of a

virulent local isolate (Xt-5) grown on potato sucrose agar medium and ten days after inoculation, individual plants were scored by employing the scale proposed by Shekhawat *et al.*<sup>1</sup>.

The  $F_1$ s of all the crosses were susceptible indicating that susceptibility was dominant. All the crosses involving resistant and susceptible parents segregated in the ratio of 63 (susceptible) : 1 (resistant) in the  $F_2$  generation indicating the presence of three pairs of independent recessive genes conferring resistance to the disease (Table I) whereas the  $F_2$  population of the cross TKM-6 (susceptible)  $\times$  IR-8 (susceptible) were all susceptible. The absence of any reciprocal differences in crosses involving resistant and susceptible parents suggested the lack of any specific role of cytoplasm in the inheritance of this character. Study of  $F_3$  generation further confirmed the  $F_2$  segregation pattern thereby indicating that three pairs of inde-

**TABLE I**  
*Segregation pattern in  $F_2$  generation*

| Cross               |          | Susceptible | Resistant | Total | Ratio obtained | $\chi^2$ | <i>p</i> value |
|---------------------|----------|-------------|-----------|-------|----------------|----------|----------------|
| BJ-1 $\times$ TKM-6 | Observed | 382         | 4         | 386   | 63 : 1         | 0.68     | 0.50-0.30      |
|                     | Expected | 380         | 6         | 386   |                |          |                |
| TKM-6 $\times$ BJ-1 | Observed | 321         | 7         | 328   | 63 : 1         | 0.81     | 0.50-0.30      |
|                     | Expected | 323         | 5         | 328   |                |          |                |
| BJ-1 $\times$ IR-8  | Observed | 461         | 6         | 467   | 63 : 1         | 0.14     | 0.80-0.70      |
|                     | Expected | 460         | 7         | 467   |                |          |                |
| IR-8 $\times$ BJ-1  | Observed | 419         | 11        | 430   | 63 : 1         | 2.32     | 20-0.10        |
|                     | Expected | 423         | 7         | 430   |                |          |                |
| TKM-6 $\times$ IR-8 | Observed | 436         | 0         | 436   | ..             | ..       | ..             |

**TABLE II**  
*Nature of segregation in  $F_3$  generation*

| Cross               |          | No. of families breeding true for S | No. of families segregating with |            |            | Total | $\chi^2$ | <i>p</i> value |
|---------------------|----------|-------------------------------------|----------------------------------|------------|------------|-------|----------|----------------|
|                     |          |                                     | 3 S : 1 R                        | 15 S : 1 R | 63 S : 1 R |       |          |                |
| BJ-1 $\times$ TKM-6 | Observed | 28                                  | 3                                | 12         | 7          | 50    | 0.80     | 0.90-0.80      |
|                     | Expected | 29                                  | 5                                | 10         | 6          | 50    |          |                |
| TKM-6 $\times$ BJ-1 | Observed | 27                                  | 4                                | 11         | 6          | 48    | 0.68     | 0.90-0.80      |
|                     | Expected | 28                                  | 5                                | 9          | 6          | 48    |          |                |
| BJ-1 $\times$ IR-8  | Observed | 24                                  | 3                                | 12         | 5          | 44    | 2.57     | 0.50-0.30      |
|                     | Expected | 26                                  | 4                                | 8          | 6          | 44    |          |                |
| IR-8 $\times$ BJ-1  | Observed | 30                                  | 4                                | 8          | 6          | 48    | 0.45     | 0.95-0.90      |
|                     | Expected | 28                                  | 5                                | 9          | 6          | 48    |          |                |

R = Resistant.

S = Susceptible.

pendent recessive genes are responsible for resistance to bacterial leaf streak disease (Table II) which may tentatively be designated as  $xt_1$ ,  $xt_2$  and  $xt_3$ .

Thanks are due to Dr. S. Y. Padmanabhan, Director and Dr. N. K. Chakrabarti, Head, Division of Plant Pathology, for providing facilities and their keen interest in the study.

Central Rice Research Institute, P. NAYAK.  
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March 10, 1975. R. N. MISRA.

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### TERFEZIA TERFEZIOIDES—A NEW RECORD FOR INDIA

DURING the collection trip of Gasteromycetes in September, 1966, the author collected a member of the order Tuberales in sandy soil where a number of fruits of *Simblum periphragmoides* Klotzsch were also growing. In India only two species of this group, viz., *Tuber indicum* Cooke and Massee (Cooke, 1892) collected by Duthie from Mussoorie hills and the second one is that of S. R. Bose who in 1948 published an account of the species without a specific name. Asci, ascospores and description of the ascocarp of the present material agree with those of *Mattirolomyces terfezioides* (Matt.) Fischer to which it is referred. Trappe (1971), however, treated *Mattirolomyces* Fischer as a sub-genus of *Terfezia* (Tul. and Tul.) Tul. and Tul. and transferred this species as *Terfezia terfezioides* (Matt.) Trappe. The fungus is being reported for the first time from India. Its illustrated account has been provided in the present note. Material studied has been deposited in Banaras Hindu University Plant Pathology Herbarium, abbreviated as BHUPP.

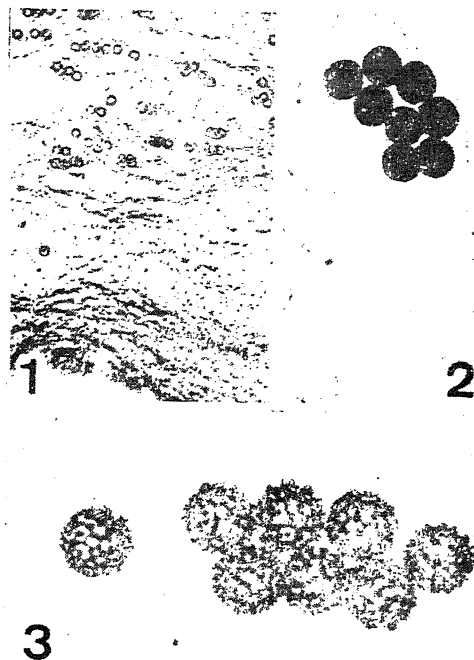
*Terfezia terfezioides* (Matt.) Trappe, *Trans. Brit. Mycol. Soc.*, 57, 91, 1971.

≡ *Choiromyces terfezioides* Matt., *Memorie Accad. Sci. Torino* (Sér. 2), 38, 384, 1887.

≡ *Mattirolomyces terfezioides* (Matt.), Fischer, *Engler Nat. Pflanzenfam.* 5 B (8), 40, 1938.

Ascocarp burried in the soil, 2–3.5 cm in diam, subglobose, attached to the substratum by a white cord-like root; peridium pallid to yellowish white, smooth, darkening with the age; gleba solid, completely fertile, aromatic, giving the sweet odour, yellow, on drying becoming grey; outer layer (Fig. 1) of thin walled, angular to elongated cells, cells 5–30  $\mu$  in diam; asci (Fig. 2) thin walled,

pseudoamyloid when young otherwise non-amyloid, ovoid to broadly clavate, without a distinct base, 4–8 spored, 100–160  $\times$  40–65  $\mu$  in size; ascospores (Fig. 3) pale brown, spherical, reticulate, meshes stout, more or less even, with a central large gutta in young spores, biserial or irregularly arranged, 18.5–24  $\mu$  in diam.



FIGS. 1–3. *Terfezia terfezioides*. Fig. 1. T.S. of a part of an ascocarp showing tissue structures, asci and ascospores,  $\times 75$ . Fig. 2. A single ascus with immaturated ascospores,  $\times 500$ . Fig. 3. Reticulate ascospores,  $\times 700$ .

Material studied: BHUPP 284, on sandy soil, near University Ghat, Leg. K. B. Khare, August 20, 1966.

This species is quite different in size and sculpturing of the ascospores from that described by Bose. He (*op. cit.*) described his species as having spiny spores of 10–12  $\mu$  in diam and the asci 2–4 spored only, whereas spores of this species are distinctly reticulate, larger in size and the asci are 4–8 spored.

The author is thankful to Prof. Heslop-Harrison, Royal Botanic Gardens, Kew, for his comments on the identification of the present species and to Prof. M. S. Pavgi, Head, Department of Mycology and Plant Pathology, Faculty of Agriculture, Banaras Hindu University, for having provided the laboratory facilities. He wishes to acknowledge the help of C.S.I.R., New Delhi, for appointing

him in the Scientist's Pool, during tenure of which this note was prepared.

Department of Mycology and K. B. KHARE,  
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Varanasi 221005, February 6, 1975.

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**NECTRIA HAEMATOCOCA—A PERFECT STATE  
OF FUSARIUM SOLANI (MART.) SACC. ON  
SOYBEAN FROM INDIA**

DURING the screening of one thousand seven hundred and fifty-nine germ plasm lines and varieties of soybean in 1969–73, several diseased specimens were examined and found affected with seedling rot at I.A.R.I. farm, New Delhi. The causal organism was identified as *F. solani*. The perfect state of the above fungus was also observed for the first time from India. The losses due to *F. solani* are approximately 7–8% of the total crop grown under naturally infected epiphytotic field conditions.

*Nectria haematococa* Berk & Br.

**Conidial state.**—Colonies on PDA yellowish, floccose, greyish white with a pale greenish yellow tinge (Ridgeway plate No. XLVI-21, V-f). Microconidia numerous, hyaline, thick-walled, allantoid to oval,  $8-16 \times 2-4 \mu$  in diam. formed on lateral conidiophores, initially made up of elongated lateral phialides. Phialides  $40-80 \times 2.5-3 \mu$  in diam. Macroconidia fusoid,  $3-6 \mu$  wide with a thick wall and a rounded well marked foot cell. The apical cell is pointed and somewhat beaked, three to five septate.

One septate =  $15 \times 3.3 \mu$ .

Three septate =  $35-46 \times 3-5 \mu$ .

Five septate =  $44-56 \times 4-5 \mu$ .

Chlamydospores abundant, globose to oval smooth,  $9-12 \times 8-10 \mu$ , terminal or intercalary.

**Perithecial state.**—Perithecia observed at  $28^\circ \text{C}$ , irregularly globose, pale orange to ochraceous at maturity, light brown and appear gelatinous with a roughly warted to furfuraceous outer wall. The outer wall is light coloured than the underlying cells. Perithecia ostiolate,  $110-250 \mu$ , thin-walled; asci cylindrical, clavate, with rounded apex and a central pore  $60-80 \times 8-12 \mu$ ; ascospores eight in a single ascus, ellipsoid to obovate,  $10.5-18 \times 4.5-5 \mu$ , hyaline at maturity, becomes light brown, slightly constricted at the single central septum and develop longitudinal striations (Fig. 1).

The above description of the species tallies with the original description as outlined by Booth<sup>1</sup> in his book.

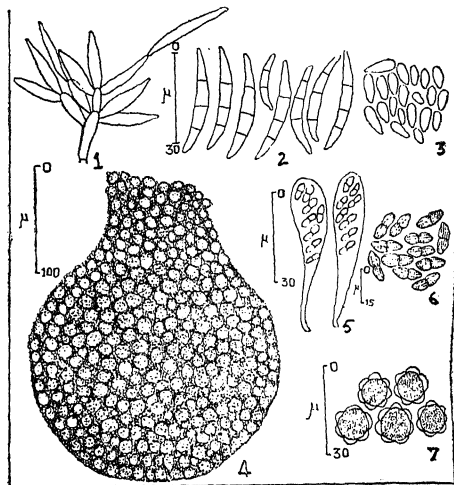


FIG. 1. *F. solani*. 1, Phialides; 2, Macroconidia; 3, Microconidia; 4, Perithecia; 5, Asci; 6, Ascospores; 7, Chlamydospores.

Culture deposited at the Indian Type Culture Collection as No. 1804, Mycology and Plant Pathology Division, I.A.R.I., New Delhi.

Apart from the above species *Fusarium graminearum*, *Nigrospora oryzae*, *Cladosporium herbarum*, *Cladosporium cladosporioides*, and *Helminthosporium rostratum* were also invariably isolated from the various parts of soybean plant. All these fungi are new records for soybean.

Thanks are due to Dr. W. Gerlach, Director, Institute of Microbiology, Berlin, West Germany, for his valuable advice and confirmation of the isolate.

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**INTERGENOMIC PAIRING IN BBCC AND CCDD  
SPECIES OF ORYZA**

THE genus *Oryza* L., ser. *Latifoliae*, consists of diploid ( $2n = 24$ , 12 II) as well as tetraploid ( $2n = 48$ , 24 II) species. The genomic constitution of the former is known to be BB and CC and the latter BBCC and CCDD<sup>1-4</sup>. Though a diploid DD species is still unidentified, it is generally inferred to belong to ser. *Latifoliae*<sup>5-6</sup> only. A better understanding of the interrelationships of the three genomes, B, C and D, is expected to help identify

the DD species, if still existent. One of the approaches would be to study meiosis in BBCC and CCDD species and infer intergenomic relationships from their pairings, if any. A comparative study of the pairing of chromosomes at first division of meiosis in these species was, therefore, considered desirable.

In the present study, meiosis was studied in five species (*O. minuta* Presl., *O. schweinfurthiana* Prod., *O. malampuzhaensis* Krishn. et Chandr., *O. latifolia* Desv., and *O. alta* Swallen) at diakinesis stage. For fixing, acetic alcohol (3:1) and for staining acetocarmine were used. The data are presented in Table I. In general, 24 II were observed in the majority of the cells of most of the species. Occasionally quadrivalents or univalents were observed.

TABLE I  
Chromosome pairing in five tetraploid ( $2n = 48$ ) *Oryza* species

| Species                    | Genome | No. of<br>PMC's<br>studied | Configuration |                 |                 |                 |                | Range |       |     | Mean |       |      |
|----------------------------|--------|----------------------------|---------------|-----------------|-----------------|-----------------|----------------|-------|-------|-----|------|-------|------|
|                            |        |                            | 24 II         | 22 II +<br>1 IV | 20 II +<br>2 IV | 18 II +<br>3 IV | 23 II +<br>2 I | IV    | II    | I   | IV   | II    | I    |
| <i>O. malampuzhaensis</i>  | BBCC   | 62                         | 29            | 17              | 14              | 2               | ..             | 0-3   | 18-24 | 0   | 0.82 | 22.35 | 0    |
| <i>O. schweinfurthiana</i> | BBCC   | 57                         | 44            | 8               | ..              | ..              | 5              | 0-1   | 22-24 | 0-2 | 0.14 | 23.63 | 0.18 |
| <i>O. minuta</i>           | BBCC   | 52                         | 35            | 3               | ..              | ..              | 14             | 0-1   | 22-24 | 0-2 | 0.06 | 23.61 | 0.54 |
| <i>O. alta</i>             | CCDD   | 69                         | 37            | 28              | ..              | ..              | 4              | 0-1   | 22-24 | 0-2 | 0.41 | 23.13 | 0.11 |
| <i>O. latifolia</i>        | CCDD   | 55                         | 41            | 12              | ..              | ..              | 2              | 0-1   | 22-24 | 0-2 | 0.22 | 23.53 | 0.07 |

In *O. malampuzhaensis*, 3 quadrivalents were observed in 53% of the cells. This might indicate the affinity between the two genomes (B and C) of *O. malampuzhaensis*.

In the African tetraploid species (*O. schweinfurthiana*'s *O. punctata*, *O. eichingeri*), Shama Rao and Seetharaman<sup>7</sup> reported upto 8 IV, while Bouharmont<sup>8</sup> recorded upto 4 IV in one material, and upto 8 IV in another. Hu and Chang<sup>9</sup> did not observe any quadrivalent in their study. In the present study, only one quadrivalent was observed in 14% cases. Since this species is widely distributed from tropical west Africa to Madagascar, it is probable that this material has retained genetic variability.

The Philippine tetraploid species, *O. minuta* contained mostly bivalents and 21 were recorded occasionally. This agrees with the observations of

other workers<sup>1</sup>. The present observation of 1 to 3 IV in BBCC species (*O. minuta*, *O. schweinfurthiana* and *O. malampuzhaensis*) supports Katayama's<sup>10</sup> observation of 4 II due to B-C pairing in AABC plants. Among the BBCC species, the chromosomes of the two genomes (B and C) seems to have well differentiated in *O. minuta* but less so in the other two species.

Out of the three tetraploid species (*O. latifolia*, *O. alta* and *O. grandiglumis*), of CCDD constitution, only two could be studied during this investigation. The modal pairing was 24 II, occasionally 1 quadrivalent or 2 univalents were observed in both the species. Normal pairing has been reported in these species by other workers<sup>1</sup>. Katayama<sup>10</sup>, however, observed 13 to 15 bivalents in AACD plants which indicate that 1 to 3 II could be due to C-D

pairing. This may lead one to infer that C and D genomes have less homology as compared to B and C genomes. However, studies on interspecific hybrids having genomic make-up of ACD and ECD indicate that C and D genomes can pair forming upto 11 or 12 bivalents<sup>11-13</sup>. Similarly, comparative studies of the octoploids of *O. minuta* (BBBBCCCC) and *O. latifolia* (CCCCDDDD) indicate that D genome suppresses intergenomic pairing as well as multivalent formation<sup>14,15</sup>. This is also supported by Hu's<sup>16</sup> observation of mostly univalents in a CCDD interspecific hybrid. It is, therefore, probable that the absence of quadrivalents in CCDD species might not be due to lack of homology between C and D genomes but due to suppression of intergenomic pairing as well as of multivalent formation by D genome.

Thanks are due to Dr. S. D. Sharma, Botanist (Rice), for providing the material and to



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for facilities to carry out this research.

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#### PLASMODESMATA IN THE EPIDERMIS OF SOME CUCURBITACEOUS FRUITS

THE plasmodesmata are not reported in the fruit wall epidermis, though their presence in other epidermal cells is quite well known<sup>1</sup>. Fine cytoplasmic connections passing through the anticlinal walls of epidermal cells are observed in the fruits of *Coccinia indica* (Fig. 1), *Luffa cylindrica* (Fig. 2, dart), *L. acutangula* var. *amara* and *Citrullus vulgaris*. In *L. cylindrica* and *L. acutangula* var. *amara* these walls are unevenly thickened. Plasmodesmata are normally present at the thinner region of the wall (Fig. 2, dart). They are present on the wall common between a subsidiary cell and an epidermal cell or two adjacent subsidiary cells in *L. acutangula* var. *amara* (Fig. 3, dart), *L. cylindrica* (Fig. 4, dart) and *C. indica*. Very minute cytoplasmic connections passing through a common wall between a guard cell and a subsidiary cell are observed in *C. indica* (Fig. 5, dart). The average number of plasmodesmata per 100 micron long cell wall is about 18

in *L. cylindrica*, 16 in *L. acutangula* var. *amara*, 22 in *C. indica* and 12 in *Citrullus vulgaris*. It is evident that the number of plasmodesmata per 100 micron long cell wall is roughly equal in *L. cylindrica* and *L. acutangula* var. *amara*. Plasmodesmata between epidermal and subsidiary cells, between two adjacent subsidiary cells and between a subsidiary cell and a guard cell indicate cytoplasmic continuity among the various epidermal components of the fruit.



FIGS. 1-5. Figs. 1 and 5, *C. indica*. Figs. 2 and 4, *L. cylindrica*. Fig. 3, *L. acutangula* var. *amara* (at darts, plasmodesmata; G, guard cell). Figs. 1-4,  $\times 360$ . Fig. 5,  $\times 660$ .

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# VOLATILE METABOLITES OF SOIL FUNGI IN RELATION TO SPORE GERMINATION AND MYCELIAL GROWTH

VOLATILE metabolites of fungi have received considerable attention in the recent years and they have been implicated in the growth and development<sup>2,3,6,8,9</sup> and ecology of soil microbes<sup>1,4,5,6,7,10</sup>. One such sphere of microbial ecology is that of soil fungistasis in which our laboratory has been keenly interested<sup>7-10</sup>.

Volatiles emitted by *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *A. terreus* Thom, *Penicillium chrysogenum* Thom, *P. jensenii* Zaleski, *P. nigricans* Bainier, and *P. notatum* Westling were studied in liquid and agar Czapek's medium employing the flask technique or chambers made of paired Petri dishes. In the former Erlenmeyer flasks of 150 ml capacity were used and 25 ml of liquid medium was dispensed in each. After autoclaving, each flask was inoculated by the spore suspension prepared from 6-8 day-old culture of soil fungi. The mouth of the flask was closed by cork plug through which a soft copper wire was pierced whose inside end was made into a loop; the loop was big enough to hold a single water agar disc (2 mm thick and 8-10 mm diam) which was exposed to the volatiles of soil fungus growing in the medium for 10 days. For germination study, the exposed agar disc was taken out for a brief period and after placement of spore suspension, was returned to its original place; germination counts for at least 200 spores were made after a further incubation of 24 hr. Effect of volatiles on spore germination and mycelial growth was also evaluated in paired Petri dish chambers<sup>2</sup>. In this technique, soil fungi were grown in Czapek's agar medium for a period of 10 days. The upper lid was then replaced by another lid of the same size which also contained agar medium and a centrally-placed inoculum disc of the test fungus or spores. The radial mycelial growth was recorded after a further incubation of 6 days. In the control set, the lower lid contained uninoculated medium. The two lids of Petri dish were sealed off, using cello tape to avoid any outward diffusion of the volatiles. Test fungi included, *Alternaria tenuis* Nees, *Curvularia geniculata* (Tracy and Earle) Boedijn, *Helminthosporium rostratum* Drechsler, and *Pestalotia* sp.; all the four are common soil inhabitants. All manipulations were made under aseptic conditions and experiments were run in triplicate at least.

Inhibition of spore germination was quite marked in the case of *A. fumigatus* and *A. terreus* and the values ranged between 50-90% (Fig. 1); spores of *Pestalotia* were, however, inhibited to a smaller

degree against these two soil fungi. *Aspergillus niger* and *A. flavus* were not as effective as the other two species. These observations are in agreement with those of Johri and Singh<sup>7</sup>. All the four species of *Penicillium*, on the other hand, could inhibit spore germination of *Alternaria*, *Curvularia*, and *Helminthosporium* to a marked degree (60-90%). *Penicillium jensenii* was the most active producer of volatiles, since this organism inhibited 80-90% of the test spores; our experience with this fungus has shown that it is an equally potent producer of non-volatile inhibitors of spore germination. As noted against species of *Aspergillus*, spore germination of *Pestalotia* was also not strongly inhibited by

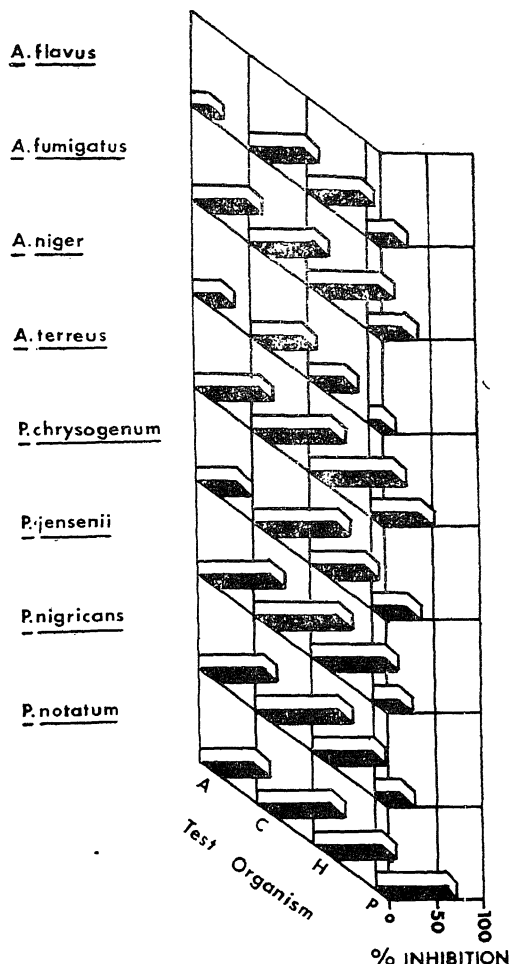


FIG. 1. *In vitro* production of volatile inhibitors by soil fungi in liquid Czapek's medium. Erlenmeyer flask-method was used for assessing the spore germination of test fungi. In this and the figures which follow, A, C, H, P, denote *Alternaria*, *Curvularia*, *Helminthosporium*, and *Pestalotia*.

these four *Penicillia*; *P. notatum* alone suppressed spore germination to a marked degree (80%).

Soil colonization studies have shown that *A. fumigatus* is the most dominant fungus in local soils and, therefore, an experiment was run in which sodium nitrate in Czapek's medium was replaced by an equivalent amount of nitrogen in the form of asparagine, ammonium chloride, ammonium nitrate, potassium nitrate, sodium nitrite, and sodium nitrate. In general it was noted that volatile production was directly proportional to the quantity of nitrogen utilized. Thus, asparagine and sodium nitrite supported good mycelial growth and volatile production of *A. fumigatus* (Fig. 2); growth was poor on ammonium chloride, ammonium nitrate, and potassium nitrate and consequently the levels of volatiles were low resulting in poor inhibition of spore germination.

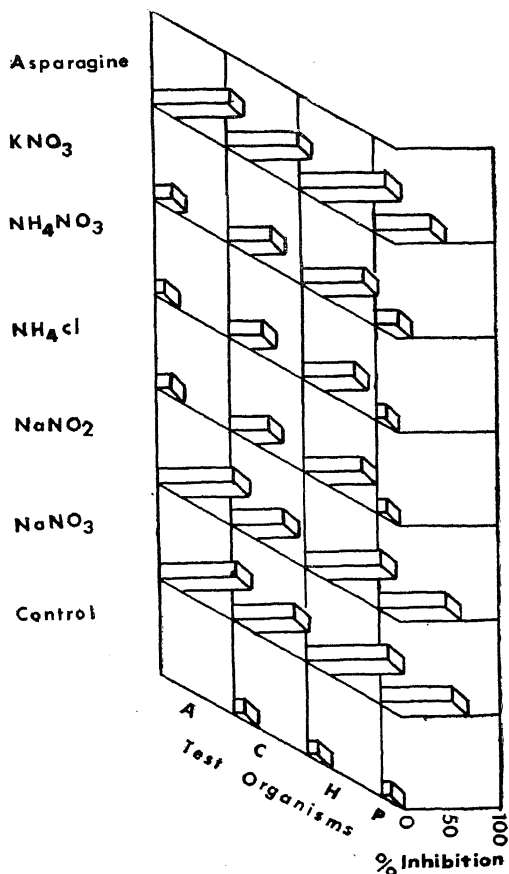


FIG. 2. *In vitro* evaluation of the effect of various nitrogenous sources on the production of volatile inhibitors by *Aspergillus fumigatus*. Nitrogen was added at a concentration equivalent to that of 2 g sodium nitrate. Control flask did not receive any nitrogenous substrate.

The release of volatiles in agar medium was comparable to that noted in liquid medium (Fig. 3). *Aspergillus fumigatus* and *Penicillium jensenii* dominated over other members as inhibitors of spore germination of *Alternaria*, *Curvularia*, and *Helminthosporium*. In contrast to its behaviour under liquid culture conditions, *P. nigricans* was quite effective in emitting volatiles in agar medium; spore germination of *Pestalotia* was once again least affected. The chief difference between the release of volatiles in liquid and agar medium was the comparatively low inhibition recorded in the latter.

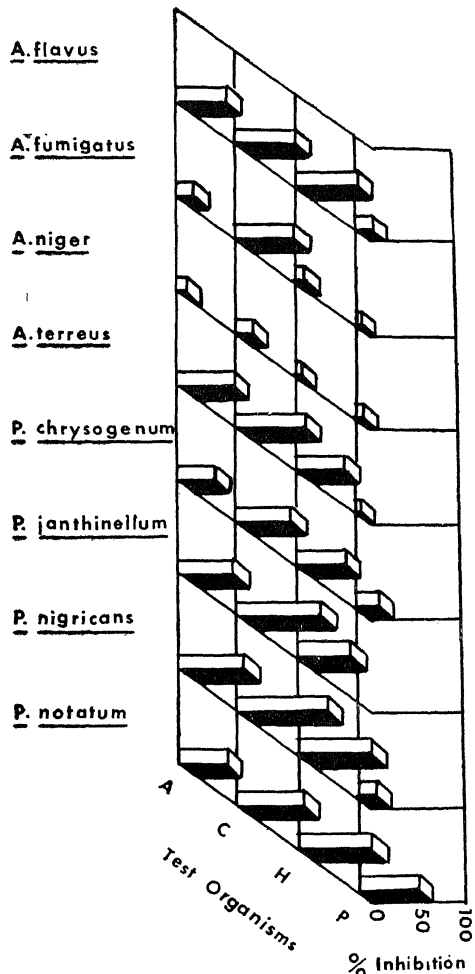


FIG. 3. *In vitro* production of volatile inhibitors of spore germination by soil fungi on agar medium. Paired Petri dish chambers were used for this experiment. Details in the text.

The effect of volatiles on mycelial growth of test fungi was less feeble than the inhibition of spore germination. Radial growth of *Curvularia* alone was

reduced to an appreciable extent (Table I); even for this test organism, only *A. fumigatus* and

TABLE I  
Effect of volatile inhibitors on the mycelial growth of test fungi

| Soil fungi                   | Radial mycelial growth (mm) |                   |                         |                   |
|------------------------------|-----------------------------|-------------------|-------------------------|-------------------|
|                              | <i>Alternaria</i>           | <i>Curvularia</i> | <i>Helminthosporium</i> | <i>Pestalotia</i> |
| Control (Uninoculated agar)  | 80                          | 70                | 70                      | 85                |
| <i>Aspergillus flavus</i>    | 75                          | 65                | 55                      | 80                |
| <i>A. fumigatus</i>          | 70                          | 60                | 45                      | 75                |
| <i>A. niger</i>              | 75                          | 70                | 60                      | 85                |
| <i>A. terreus</i>            | 75                          | 70                | 55                      | 85                |
| <i>Penicillium nigricans</i> | 75                          | 65                | 50                      | 80                |
| <i>P. notatum</i>            | 70                          | 65                | 50                      | 80                |
| <i>P. chrysogenum</i>        | 75                          | 70                | 55                      | 85                |
| <i>P. jenseni</i>            | 65                          | 60                | 45                      | 75                |

Soil fungi were grown in Czapek's agar medium for 10 days in chambers made of paired Petri dishes; the growth of test fungus was measured 6 days after placement of the inoculum disc.

*P. jenseni* could inhibit mycelial growth to an extent of 30–40%. Some inhibition of mycelial growth of *Alternaria*, *Helminthosporium*, and *Pestalotia* was also noted but the values were considerably low (5–10%).

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## SHORT SCIENTIFIC NOTES

### A New Species of *Acrosporium* Nees ex Gray with a Note on *Oidium pedilanthi* Mathur et al.

During survey of plant pathogenic fungi in and around Jabalpur, M.P., in 1973 and 1974 the authors came across a powdery mildew on the leaves of *Scoparia dulcis* L. Mildew appears on both the sides of leaves predominantly on upper side. Gradually necrosis develops in affected parts and leaves defoliate. The pathogen was identified as *Acrosporium* sp. We feel considerable difficulty while disposing-off this collection of *Acrosporium* under the known species, because cleistothecia were not observed in the collection, whereas the most useful classifications are based on their cleistothecial states<sup>1,5</sup>. Moreover in *Acrosporium* the delimitation of species is based largely and primarily on the host plant attacked<sup>8</sup>. So far there is no record of any species of it on *Scoparia* or any other member of the

family Scrophulariaceae<sup>2,4,9</sup>. It is, therefore, proposed to report the present fungus as a new species.

The specimen has been deposited in the herbarium of Department of Plant Pathology, J.N. Agricultural University, Jabalpur.

*Acrosporium scopariae* sp. nov.

Colonies sparse; mycelium superficial, branched, hyaline, unequal in thickness, haustoria globose; upto 5  $\mu$  wide; conidiophores simple, erect, clavate, upto 6-septate, 60–120  $\times$  8–11  $\mu$ , conidia hyaline, granulated internally, oval to elliptical, 1-celled, usually in chains of 3–4, 25–37  $\times$  12–19  $\mu$ .

On leaves of *Scoparia dulcis* L. (Scrophulariaceae) Experimental Fields, Agric. Univ. Adhartal, Jabalpur, December, 1973, Leg. N. D. Sharma, H. P. P. INKVV No. 15.

Colonies sparsus, mycelium superficialis, ramosum, hyalina, diametro inaequalibus, haustoriis globosis, usque  $5\ \mu$  crassae, conidiophores simplicibus, erectae, clavatus, usque 6-septatis,  $60\text{--}120 \times 8\text{--}11\ \mu$ ; conidiis hyalina, interdum granulosae, ovalis vel elliptica, semel cellularis, plerumque 3-4 catenulatus.

Habit: in foliis viventibus *Scoparia dulcis* L. (Scrophulariaceae), Experimental Fields, Agric. University, Adhartal, Decembri 1974, Leg. N. D. Sharma, H. P. P. JNKVV No. 15.

While scrutinising the literature on *Acrosporium* (= *Oidium*) we have noticed a new species of *Oidium* recorded by Mathur *et al.*<sup>3</sup> on *Pedilanthus tithymaloides* Poit (Euphorbiaceae). Rao<sup>5</sup>, recorded *Sphaerotheca euphorbiae* (Cast) Sal. on this host from Hyderabad and more recently it has been recorded by Sharma and Jain<sup>7</sup> along with a hyper-parasite *Cicinobolus cesatii* de Bary parasitizing this mildew. We have checked conidial state of *Sphaerotheca euphorbiae* of our collection with that of *Oidium pedilanthi* Mathur *et al.*, and have found that both of them are the same fungus.

Therefore, the name of the mildew commonly occurring on *Pedilanthus tithymaloides* should be as follows:

*Sphaerotheca euphorbiae* (Cast) Salm.

= *Oidium pedilanthi* Mathur *et al.*, *Indian Phytopathol.*, 1971, 24, 62.

Department of Plant Pathology, N. D. SHARMA.  
J.N. Krishi Vishva Vidyalaya, A. C. JAIN.  
Jabalpur-4, March 7, 1975.

### *Ophioglossum vulgatum* Linn.—A New Record from Manipur

During April 1972, one of us (N. I. S.), while searching for young seedlings of *Helminthostachys zylanica*, came across a species of *Ophioglossum* in the low lying grazing group of Uchekon Khunou, Central Manipur District, along with *Imperata cylindrica* (Poaceae) in the rainy season. The species, after critical examination, was found to be *Ophioglossum vulgatum*, which is a new record for the state of manipur<sup>1-3</sup>, and conforms<sup>4-10</sup> to the following description.

Plant erect, 10 cm long; rhizome cylindrical, erect, producing a large number of adventitious roots. Sterile lamina ovate; venation reticulate, mid-vein right upto the apex; aerioles broad with "venatio anaxeti". Stomata lie parallel with occasional irregularity, size on the lower surface  $110.1 \times 56.4\ \mu$ . Epidermal cells dorsal surface  $237.6 \times 60.6\ \mu$  in size, ventral surface  $224.4 \times 57.9\ \mu$  in size. Attachment of the fertile spike above the middle. Sporangia 19-33 per spike. Spores spherical, exine  $1.5\text{--}2\ \mu$  in thickness, occasional triradiate mark, occasional perillate outgrowth,  $48\text{--}57\ \mu$  in diameter. Rhizome ectophloic siphonostele (meristele number varies 3-4), leaf, trace 1-2, root trace present.

Department of Botany, NAMEIRAKPAM I. SINGH.  
St. Edmund's College, D. CHOUDHURY.  
Shillong 793003, April 22, 1975.

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## INFORMATION TO CONTRIBUTORS

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# PREDICTION OF $\beta$ -REGIONS IN GLOBULAR PROTEINS

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## ABSTRACT

A method for predicting  $\beta$ -regions in globular proteins using the one-dimensional Ising model theory, is presented. The parameters used have been obtained from published statistical data on the potential of each amino acid residue to be in the  $\beta$ -structure. The results obtained for five globular proteins of known secondary structures, show an average accuracy of 70% for the prediction of  $\beta$ -residues and 84% for predicting  $\beta$ -regions.

## INTRODUCTION

A GREAT deal of effort has recently been made to predict the secondary structures in globular proteins from their amino acid sequences<sup>1</sup>. We present here a new method for predicting the  $\beta$ -regions in globular proteins. The method makes use of the fact that individual amino acid residues can be ascribed, what may be called, a ' $\beta$ -structural potential', which does not depend on its neighbours along the polypeptide chain, so that it should be possible to use a one-dimensional Ising model approach, similar to the one employed in the helix-coil transition theories, for treating the equilibrium between the  $\beta$ - and coil-states of the residues. The prediction method utilises the Lifson-Roig formalism of the conformational transitions in polypeptides<sup>2</sup>. Recently, Froimowitz and Fasman<sup>3</sup> have used this theory for predicting the  $\alpha$ -helical regions of globular proteins. Our prediction procedure employs this theory for the first time to identify the  $\beta$ -regions in these proteins.

The results obtained by using the theory on five globular proteins, whose secondary structures are known by x-ray diffraction studies, indicate the average accuracy of our method to be 70% for the prediction of  $\beta$ - and coil residues and 84% for predicting  $\beta$ -regions.

## METHODS

For our purpose in this paper, we employ the term  $\beta$ -residue to denote the amino acid residue having its dihedral angle  $\Psi$  and  $\phi$  close to  $135^\circ$  and  $-143^\circ$ , respectively. A  $\beta$ -region is defined as consisting of at least three  $\beta$ -residues in succession along the direction of the polypeptide chain. The term  $\beta$ -structure is treated as being synonymous with  $\beta$ -region without any reference to its hydrogen-bonding properties.

Our two-state ( $\beta$ - or coil) model does not include the  $\alpha$ -helical state of the amino acid residues. The co-operativity and propagation parameters,  $v$  and  $w$  (in the Lifson-Roig notation), used in the treatment of conformational transitions, are normally obtained from the experimental data on polypeptides. While these parameters for the helix-coil transition are available for several amino acid residues, no such data is available for the  $\beta$ -coil transition. However, from a statistical analysis of

the data available on several globular proteins whose structures have been solved by X-ray crystallography, Chou and Fasman<sup>4</sup> have obtained a set of empirical values for all the amino acid residues in the  $\beta$ -region; these ' $\beta$ -structural potential' values have been used by us as the equivalents of  $v$  and  $w$ . However, the parameter  $v$  is divided into  $v_{N,\beta}$  and  $v_{\beta,c}$ , which are proportional to the frequencies of occurrence of a  $\beta$ -residue, respectively, at the N- and C-terminal ends of a  $\beta$ -region. (The proportionality factor has arbitrarily been taken to be 0.5.) The parameter  $w$ , denoted here onwards as  $w_\beta$ , is taken as the ratio of the frequency of occurrence of a given residue in the middle of a  $\beta$ -region over its frequency of occurrence in the coil state. The values of  $v_{N,\beta}$ ,  $v_{\beta,c}$  and  $w_\beta$  are given in Table I.

TABLE I

*Values of the conformational parameters employed\**

| Amino acid | $w_\beta$ | $v_{N,\beta}$ | $v_{\beta,c}$ |
|------------|-----------|---------------|---------------|
| Gly        | 0.207     | 0.024         | 0.026         |
| Ala        | 0.535     | 0.040         | 0.038         |
| Val        | 0.911     | 0.080         | 0.064         |
| Leu        | 0.672     | 0.031         | 0.059         |
| Ile        | 0.757     | 0.062         | 0.057         |
| Ser        | 0.208     | 0.027         | 0.027         |
| Thr        | 0.416     | 0.036         | 0.029         |
| Met        | 1.000     | 0.072         | 0.054         |
| Pro        | 0.155     | 0.030         | 0.018         |
| Phe        | 0.581     | 0.055         | 0.043         |
| Tyr        | 0.393     | 0.065         | 0.055         |
| Asp        | 0.263     | 0.032         | 0.032         |
| Asn        | 0.181     | 0.027         | 0.030         |
| Glu        | 0.109     | 0.001         | 0.018         |
| Gln        | 0.571     | 0.069         | 0.021         |
| His        | 0.281     | 0.007         | 0.021         |
| Lys        | 0.256     | 0.023         | 0.035         |
| Arg        | 0.273     | 0.007         | 0.045         |
| Trp        | 0.529     | 0.080         | 0.012         |
| Cys        | 0.444     | 0.019         | 0.047         |

\* Computed from data of Chou and Fasman<sup>4</sup>.



TABLE II  
Comparison of observed and predicted  $\beta$ -regions

| Protein<br>Chain length             | $\beta$ -regions   |   | % of<br>residues<br>predicted<br>correctly | % of<br>$\beta$ -residues<br>predicted<br>correctly | % of<br>$\beta$ -regions<br>predicted<br>correctly |
|-------------------------------------|--|---|--|---|--|
|                                     | Experimental<br>(X-ray*)   | Predicted   |  |   |  |
| Concanavalin-A<br>(239)             | 4-9, 25-29, 48-55,<br>59-66, 73-78, 92-97,<br>106-116, 125-132,<br>140-144, 173-177,<br>190-199, 209-215 | 3-7, 25-29, 41-43, 52-54,<br>59-66, 88-93, 106-108,<br>126-134, 140-144, 174-178,<br>188-192, 210-215, 230-235  | 74   | 63  | 83   |
| Staphylococcal<br>nuclease<br>(149) | 12-19, 22-27, 30-41,<br>71-75, 87-94,<br>110-114   | 13-18, 22-27, 31-40, 89-94,<br>102-105, 107-115, 123-125  | 86   | 76  | 83   |
| Subtilisin<br>BPN<br>(275)          | 28-32, 45-50, 89-94,<br>120-124, 148-152,<br>174-180, 205-209  | 26-32, 71-75, 89-96,<br>106-108, 119-124, 142-144,<br>146-153, 174-180, 198-200,<br>230-235, 245-247, 261-263,<br>268-273   | 80   | 72  | 86   |
| Lactate<br>dehydrogenase<br>(330)   | 23-27, 49-53, 72-75,<br>77-81, 93-97, 134-137,<br>188-192, 285-289,<br>291-294, 301-307                  | 23-34, 39-44, 48-55, 62-64,<br>70-72, 76-79, 91-96,<br>120-122, 172-174, 184-191,<br>200-204, 253-264, 270-275,<br>284-287, 290-292, 301-306,<br>313-316, 322-326 | 75   | 71  | 80   |
| Ferredoxin<br>(54)                  | 1-5, 27-32   | 2-5, 20-24, 27-32   | 89   | 91  | 100  |
| Average                             | ..   | ..  | 78   | 70  | 84   |

\* X-ray data have been taken from published literature.

The probability of a given residue, occurring at position  $i$  in the polypeptide chain of  $N$  residues, to be in the  $\beta$ -region is obtained as (see references 3 and 5):

$$P_{\beta}(i) = (0, 0, 1) \begin{bmatrix} i-1 \\ \pi \\ z=1 \end{bmatrix} W(k) \begin{bmatrix} w_{\beta} & v_{\beta, c} & 0 \\ 0 & 0 & 0 \\ v_{\alpha, \beta} & v_{\alpha, \beta} & v_{\beta, c} \end{bmatrix} \begin{bmatrix} N \\ \pi \\ k=i+1 \end{bmatrix} W(k) \begin{bmatrix} 0 \\ 1 \\ 1 \end{bmatrix} / Z$$

where  $W$  and  $Z$  are, respectively, the statistical weight matrix and partition function of the Lifson-Roig formulation and  $k$  is the index of the position of the amino acid residues in the chain. The average  $P_{\beta}$  for all residues  $\langle P_{\beta} \rangle$  was used for identifying whether a given residue would be in a  $\beta$ -region or coil region.

The accuracy of our prediction method was evaluated as:

Per cent of residues  
(or regions) correct =  $100(N - \text{number incorrect})/N$ .

where  $N$  is the total number of  $\beta$ -residues (or  $\beta$ -regions) or is the total number of both  $\beta$ - and coil residues, as the case may be.

#### RESULTS AND DISCUSSION

Subtilisin BPN', staphylococcal nuclease, lactate dehydrogenase, concanavalin-A and ferredoxin have

been selected as representatives of different classes of globular proteins for the purpose of our prediction method. The chain lengths of these proteins range from 54 to 330, while the percentages of  $\beta$ -structure in them as determined by the x-ray method vary from 10 to 38%.

Table II summarizes the results obtained by us for these five proteins. On the whole, 78% of all (i.e.,  $\beta$ - and coil) residues, and 70%  $\beta$ -residues, have been predicted correctly. In addition, 84% of all  $\beta$ -regions have been correctly predicted. These values are in fact better than those obtained from some of the more elaborate predictive methods (see Burgess *et al.*<sup>1</sup>, for a comparative evaluation of prediction methods for  $\beta$ -structure).

It may be observed from Table II that a large number of  $\beta$ -regions have been correctly identified by our procedure. There are several over-predictions of  $\beta$ -residues and  $\beta$ -regions. Many of the over-predicted regions are, however, found to occur in the observed helical regions or in  $\beta$ -turns<sup>1-4</sup> (not shown in Table II). The over-prediction of  $\beta$ -regions is a natural consequence of our two-state model.

The results obtained by us give strong support to the validity of the one-dimensional approach in treating the  $\beta$ -structure. It is pertinent to observe here that, while the conformational parameters of the amino acid residues were obtained statistically from data on the *three-dimensional* conformations of the globular proteins and could, therefore, be expected to incorporate long-range interactions also in them, their actual usage in predicting the conformation of a protein with an unknown structure by the use of many of the predictive methods<sup>1</sup>, involves a *one-dimensional* approach, since the protein polypeptide chain is followed *sequentially and linearly* from one end to the other, *including only near-neighbour interactions*. This provides the rationale for the success of our one-dimensional Ising model approach for  $\beta$ -structure prediction.

Such a model was not hitherto attempted owing to the long-range nature of the formation of this structure. Thus, for example, Froimowitz and Fasman<sup>5</sup> observe that "it is not at all clear at present how to incorporate the  $\beta$ -state into the model". It should, however, be pointed out, that the application of the Lifson-Roig formalism for the prediction of the  $\beta$ -structure does *not* mean that the one-dimensional *physical* theory of formation of  $\beta$ -structure is implied, since this structure is a two-dimensional one in principle. It only emphasizes the importance of short-range interactions in governing the secondary structure of globular proteins, which has been amply documented<sup>6,7</sup>.

We are currently working on the application of our method to several other proteins and also on the simultaneous prediction of both  $\alpha$ -helix and  $\beta$ -structure in these proteins using the one-dimensional approach.

#### ACKNOWLEDGEMENT

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## STUDIES ON 2, 4-DIHYDROXYVALEROPHENONE OXIME

## Part IV. Spectrophotometric Determination of Iron (III)

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## ABSTRACT

Iron (III) forms purple coloured stable complex with 2, 4-dihydroxyvalerophenone oxime having  $\lambda_{\text{max}}$  at 510 nm at pH range 2.0–3.0. The composition of the complex (metal : ligand = 1 : 1) has been determined by continuous variation, the molar ratio, the slope ratio and the Bent and French's methods. Beer's law is obeyed between an iron concentration of 1.0 to 56.0 ppm. The molecular extinction coefficient of the complex is  $9.0 \times 10^2$  at 510 nm and the sensitivity is 0.062 mg Fe/cm<sup>2</sup>. The stability constant of the complex at pH 2.7 has a value  $K = 1.080 \times 10^3$  and the standard free energy of formation of the complex is found to be 4.2 Kcal/mole at 30°C. Limits of interference due to the presence of foreign ions have been determined. The ligand is highly selective for the spectrophotometric determination of iron (III).

A NUMBER of reagents<sup>1-5</sup> have been used for spectrophotometric determination of iron(III). Gupta and Coworkers<sup>6-8</sup> have reported earlier, the physico-chemical studies on the chelates of Cu(II), Ni(II), VO(II) with 2, 4-dihydroxyvalerophenone oxime (DHVOX). This communication deals with the spectrophotometric studies of Fe(III)-DHVOX complexes.

## MATERIALS AND METHODS

2, 4-Dihydroxyvalerophenone<sup>9</sup> was synthesized by condensing *n*-valeric acid with resorcinol in the presence of anhydrous zinc chloride at 160°C. Its oxime was prepared by refluxing its alcoholic solution with hydroxylamine hydrochloride in the presence of anhydrous sodium acetate. A solution of the reagent in alcohol (40%) was used in all experiments. Ferric nitrate solution was analysed by standard procedure. Systronics Spectro Colorimeter (Type 103) was used for absorbance measurements. All pH measurements were made with Systronics pH meter (Type 322).

## RESULTS AND DISCUSSION

The method of Vosburg and Cooper<sup>10</sup> was employed to determine the nature and the number of complexes present in the purple solution. Mixtures containing 1 : 1, 1 : 2, 1 : 3, 1 : 4 and 2 : 1 mole ratio of iron(III) to the DHVOX were prepared keeping the total volume 12 ml in each case. The preliminary studies had shown that the intensity of the purple colour was very sensitive to hydrogen ion concentration. The pH of each mixture was, therefore, adjusted between 2.0 and 3.0. The absorbance measurements were carried out between 400 and 700 nm. The maximum absorbance of the complex was found to be at 510 nm. The maximum remains unaltered irrespec-

tive of stoichiometry of the components showing the presence of only one complex.

*Effect of pH :*

The colour of the complex was reddish brown above pH 3.1 and purple between pH 2.0 and 3.0. Above pH 3.0, the colour of the complex was highly unstable, but it was quite stable below pH 3.0. The studies were, therefore, confined to pH 2.0 to 3.0. The optimum pH range for the formation of the complex was 2.6–2.8. The coloured complex was formed instantaneously on mixing the reagents and was stable for 8 hours.

*Effect of Reagent :*

A series of solutions containing 2.0 ml of iron(III) ( $10^{-2}$  M) and varying amounts of the reagent ( $10^{-2}$  M) were prepared and the total volume made upto 50 ml, keeping the ethanol concentration at 24% in each case. The pH of the solution was kept at 2.7. The absorbance of these solutions was measured at 510 nm. It was found that 14–15 fold excess of the reagent was necessary to get the maximum colour intensity.

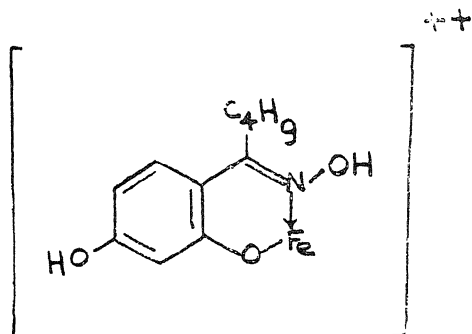
*Composition of the Complex :*

In all the measurements, requisite amounts of ethanol were added to maintain the proportion of ethanol at 24% in the final dilution. pH was adjusted to 2.7 and ionic strength was maintained at 0.1 M NaClO<sub>4</sub>. The formula of the complex was established by Job's method of continuous variation<sup>11</sup>, mole ratio method<sup>12</sup>, slope ratio method<sup>13</sup> and logarithmic method of Bent and French<sup>14</sup>. The results from all the methods indicated the formation of a 1 : 1 (metal : ligand) complex.

*Structure of the Complex :*

Assuming that the phenolic hydrogen is replaced by the ferric ion which in turn is coordinated to

nitrogen of the oximino group, the structure of the complex may be written as :



#### Stability Constant of the Complex :

The stability constant of the complex was calculated from the data in the molar ratio method, using the relation :

$$K = C(1 - \alpha)/(m\alpha C^m (n\alpha C)^n)$$

and

$$\alpha = (Em - Es)/Em$$

where  $m$ ,  $\alpha$ ,  $\eta$ ,  $C$ ,  $Em$  and  $Es$  have their usual significance. The stability constant of the complex was found to be  $1.08 \times 10^3$ . The standard

free energy of formation of the complex ( $\Delta F = -RT \ln K$ ) was found to be  $-4.2$  Kcal/mole at  $30^\circ \text{C}$ .

The iron(III) complex obeys the Beer's law over the concentration range  $1.0$  to  $56.0$  ppm of iron. From the slope ratio curve the value of molecular extinction coefficient is  $9.0 \times 10^2$  at  $510$  nm at  $\mu = 0.1$  M  $\text{NaClO}_4$ . The sensitivity is found to be  $0.062 \mu\text{g Fe/cm}^3$  (Sandell Scale<sup>15</sup>) and the optimum concentration of ferric ions is  $9-29$  ppm.

#### Effect of Diverse Ions :

The influence of foreign ions on the estimation of iron using DHVOX was also studied in the usual manner. A limit of  $2.5\%$  change in absorbance was taken as limiting concentration. It was observed that at  $11.2$  ppm of iron(III)  $1000$  ppm concentrations of  $\text{Al(III)}$ ,  $\text{Ba(II)}$ ,  $\text{Cd(II)}$ ,  $\text{Ca(II)}$ ,  $\text{Fe(II)}$ ,  $\text{Mg(II)}$ ,  $\text{Mn(II)}$ ,  $\text{Ni(II)}$ ,  $\text{Sr(II)}$ ,  $\text{UO}_2(\text{II})$ ,  $\text{Zn(II)}$ ,  $\text{NH}_4(\text{I})$ ,  $\text{Zr(IV)}$ ,  $\text{Cl}^-$ ,  $\text{ClO}_4^-$ ,  $\text{NO}_2^-$ ,  $\text{SO}_4^{2-}$ ;  $750$  ppm concentrations of  $\text{Co(II)}$ ,  $\text{Br}^-$ ;  $500$  ppm concentrations of  $\text{Cr(III)}$ ,  $\text{Ag(I)}$ ;  $150$  ppm concentrations of  $1^-$  and  $10$  ppm concentrations of  $\text{Cu(II)}$  and  $\text{Pd(II)}$  could be tolerated,  $\text{PO}_4^{3-}$ ,  $\text{F}^-$ , citrate and oxalate interfere at all levels.

A comparison of the properties of  $\text{Fe(III)}$  complexes with DHVOX and other ligands given in Table I shows that the conversion of the keto

TABLE I  
Characteristics of the ferric complexes

| Name of the complex                                   | Optimum pH | Selected pH for study | $\lambda_{\text{max}}$ nm | Molecular extinction coefficient | Stability constant $\mu = 0.1$ M $\text{NaClO}_4$ | Sensitivity $\mu\text{g/cm}^2$ (Sandell Scale) | Optimum concentration range of $\text{Fe}^{3+}$ ions ppm | $\Delta F$ KCal/mole |
|---|------------|-----------------------|---------------------------|----------------------------------|---|--|--|----------------------|
| Ferric-2,4-dihydroxy-acetophenone <sup>1</sup>        | 2.9-3.0    | 2.95                  | 470                       | $21.04 \times 10^2$              | $0.802 \times 10^3$                               | 0.084  | 5.3-18.7   | -4.000               |
| Ferric-2,4-dihydroxy-propiofenone <sup>2</sup>        | 2.9-3.0    | 2.95                  | 470                       | $13.78 \times 10^2$              | $1.29 \times 10^3$                                | 0.126  | 8.1-28.5   | -4.285               |
| Ferric-2,4-dihydroxy-butyrophenone <sup>3</sup>       | 2.9-3.0    | 2.95                  | 470                       | $12.37 \times 10^2$              | $0.68 \times 10^3$                                | 0.131  | 9.0-31.7   | -3.903               |
| Ferric-2,4-dihydroxy-propiofenone oxime <sup>4</sup>  | 2.6-2.8    | 2.7                   | 510                       | $21.74 \times 10^2$              | $1.43 \times 10^3$                                | 0.056  | 5.1-18.0   | -4.550               |
| Ferric-2,4-dihydroxy-butyrophenone oxime <sup>5</sup> | 2.6-2.8    | 2.7                   | 510                       | $15.00 \times 10^2$              | $1.1 \times 10^3$                                 | 0.074  | 7.4-26.1   | -4.288               |
| Ferric-2,4-dihydroxy-valerophenone oxime              | 2.6-2.8    | 2.7                   | 510                       | $9.00 \times 10^2$               | $1.08 \times 10^3$                                | 0.062  | 8.6-28.8   | -4.211               |

group into the ketoxime group induces a shift in the optimum pH (from 3.0 to 2.8) and a hypsochromic effect from 470 nm to 510 nm. With the increase in the number of carbon atoms in the side chain, the molecular extinction coefficient and sensitivity are found to decrease and a corresponding change is brought about in the optimum concentration range. It has been observed that the selectivity is enhanced by lengthening of the side chain. The stability constants of the ferric complexes with 2,4-dihydroxypropiofenone and its oxime are greater than those with 2,4-dihydroxyacetophenone and its oxime. This increase in the stability constant is due to the greater inductive effect of  $-C_2H_5$  group as compared to  $-CH_3$  group. On the other hand, the stability constant decreases after 2,4-dihydroxypropiofenone and its oxime. This may be due to the steric effect of the bulkier groups ( $-C_2H_5$ ,  $-C_4H_9$ ) attached to the ketonic or ketoxime group, the inductive effect of  $-C_3H_7$  or  $-C_4H_9$  group being nearly the same as that of  $-C_2H_5$  group. Moreover, the absorbance values are practically constant in the range 500 to 520 nm, giving rise to a 20 nm wide plateau, which adapts this reagent to the use of filter photometers.

These studies clearly show that 2,4-dihydroxyvalerophenone oxime is a very selective reagent for ferric ions.

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## THE EFFECT OF SOIL MOISTURE DEFICIT ON GROWTH AND ALKALOIDAL CONTENT OF *CATHARANTHUS ROSEUS* G. DON.

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## ABSTRACT

The increase of soil moisture deficit (SMD) level up to 95%, caused significant decrease in fresh and dry weights as well as the moisture content of different organs of *Catharanthus roseus* G. Don. The alkaloidal and the vincalkeboline contents of leaves, stems and roots of plants subjected to different levels of SMD were colorimetrically determined. The leaves showed the highest concentration of alkaloids at all SMD levels in comparison with stems and roots. Both leaves and stems could serve as the main plant organs for their highest content of alkaloids.

## INTRODUCTION

SOIL moisture deficit is a decisive factor in regulating growth and constituents of plants (Hsiao, 1973). Little could be traced in the literature to the effect of soil moisture deficit on the secondary plant constituents which have medicinal values. *Catharanthus roseus* G. Don (*Vinca rosea*

L.) was shown to be a source of many alkaloids, (Svoboda, 1958) possessing antitumor action (Noble *et al.*, 1958; Johnson, *et al.*, 1959). Variation of growth and alkaloidal content of this plant, as affected by growth regulators was reported (Markiewicz, 1967; Masoud *et al.*, 1968). Two questions arose: (1) Does the variation of soil moisture deficit affect the growth as well as the

distribution and accumulation of alkaloids in various parts of this plant, and (2) What is the proper and optimum level of soil moisture deficit to obtain the highest alkaloidal content. These alkaloids were determined colorimetrically as vincal leukoblastine. The latter compound represented one of the most important active alkaloids against leukemia (Johnson *et al.*, 1959).

### 1. Materials and Methods

The seeds of *Catharanthus roseus* G. Don were germinated in culture flats on 1st February 1972. Two months later, the uniform plants were transplanted to the prepared field plots. Each plot was 10 rows wide (66 cm apart) and 6 m long. The soil of the plots was Nile alluvium, clay loam in texture with fairly deep water table. The experimental design was complete randomized blocks with four replicates. After transplanting, irrigation water was added weekly to the plots. The plants were fertilized before the 3rd irrigation with the following rates per acre: 100 kg urea, 200 kg superphosphate and 30 kg potassium sulphate. After the 4th irrigation, four levels of soil moisture deficit (SMD) were adopted at the root zone for 5 months. Irrigation water was added to the plots when plants consumed 25, 50, 75 and 95% of the total available water capacity at the root zone. In order to obtain the desirable SMD level, soil samples were taken from the depths; 0-20, 20-40 and 40-60 cm for determination of soil moisture content and the corresponding values of SMD were calculated.

The plants grown in the centre of each plot (4 rows with 4 m in length) were harvested on 30th September (during flowering stage). Then, two groups of 10 plants each were randomly taken. Fresh and dry (at 105° C) weights of different plant organs (leaves, stems and roots) of the 1st group were recorded and the moisture content on dry weight basis was calculated. The plant organs of the 2nd group were dried at 70° C, powdered and stored in air-tight glass containers until used for subsequent analysis of the alkaloids.

### 2. Alkaloidal Determination

The alkaloidal concentration in the different plant organs was determined colorimetrically as vincal leukoblastine (mg/g dry weight). The powdered plant material (10 g) was thoroughly moistened with conc. ammonia, dried, and then exhaustively extracted with chloroform. The extract was concentrated, whereupon a dark oily residue resulted, which answered positively to Mayer's reagent. The oily residue was extracted repeatedly with small portions of 2% tartaric acid solution. The acid extracts were rendered weakly alkaline by the addition of dilute ammonia till pH was 9, then

extracted with pure chloroform (three aliquots), concentrated, dried over anhydrous sodium sulphate, filtered, then distilled off under reduced pressure. The alkaloidal residue was redissolved in 200 ml of A.R. chloroform.

The colour reagent was a solution of 1 g of ferric ammonium sulphate in 25 ml of distilled water, to which 75 ml of conc. sulphuric acid was added (Jakovljevic, 1964). Two ml of the chloroform solution of the alkaloid were taken and the solvent evaporated. The residue was dissolved in 2 ml glacial acetic acid and to this 2 ml of the colour reagent was added. The per cent of transmission was measured at 538 m $\mu$  after 15 minutes, using spectrophotometer (Carl Zeiss type SPEKOL).

Standard curve showing the relationship between the alkaloid content and the per cent transmission were obtained by taking a known amount (100-2500  $\mu$ g) of vincal leukoblastine and developing the colour and measuring the per cent transmission as in the case of the unknown.

The alkaloidal content was calculated as mg/g dry weight of the different plant parts. The data were statistically analyzed using Duncan's new multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### 1. Plant Growth

In general, the distribution of dry matter could be arranged in the following descending order: stems, leaves and roots for different levels of soil moisture deficit (SMD) (Table I). Both the fresh and the dry weights of leaves, stems and roots significantly decreased as SMD level at the root zone increased. Longenecker and Lysterly (1969) also found that moisture content in the leaves of cotton plant decreased as the SMD increased. From Table I, it could be concluded that 25-50% SMD level was the most efficient for increasing the dry weight of the leaves, stems and roots of *Catharanthus roseus* G. Don.

### 2. Alkaloidal Content

Figure 1 showed that the variation in alkaloidal concentration (mg/g dry weight) as vincal leukoblastine had the same trend for different levels of SMD. The quantity of the alkaloids in the leaves, stems and roots attain a maximum value between 50 and 75%. Under high level of SMD, the metabolic pathways of the plants are directed towards alkaloid production. From the present results, it could also be concluded that the leaves showed the highest accumulation of alkaloids.

In order to clarify the optimum SMD level for this plant, the alkaloidal content was calculated on the basis of the dry weight of different organs, as

TABLE I

The effect of soil moisture deficit on the weights and moisture content of leaves, stems and roots of *Catharanthus roseus* G. Don.

| Soil moisture deficit % | Leaves         |              |                     | Stems          |              |                     | Roots          |              |                     |
|-------------------------|----------------|--------------|---------------------|----------------|--------------|---------------------|----------------|--------------|---------------------|
|                         | Fresh Weight g | Dry weight g | Moisture* content % | Fresh weight g | Dry weight g | Moisture* content % | Fresh weight g | Dry weight g | Moisture* content % |
| 25                      | 205.2 a**      | 36.4 a       | 463 a               | 308.7 a        | 74.2 a       | 316 a               | 79.4 a         | 20.9 a       | 279 a               |
| 50                      | 198.9 a        | 37.0 a       | 437 a               | 287.1 b        | 73.2 a       | 292 a               | 69.6 b         | 20.0 a       | 248 b               |
| 75                      | 149.2 b        | 31.4 b       | 375 b               | 200.2 c        | 57.2 b       | 250 b               | 48.8 c         | 14.5 b       | 236 b               |
| 95                      | 83.8 c         | 20.7 c       | 304 c               | 131.7 d        | 40.9 c       | 222 c               | 29.5 d         | 9.6 c        | 207 c               |

\* Calculated on dry weight basis.

\*\* Means followed by the same letter within a column are insignificant difference at 5% level.

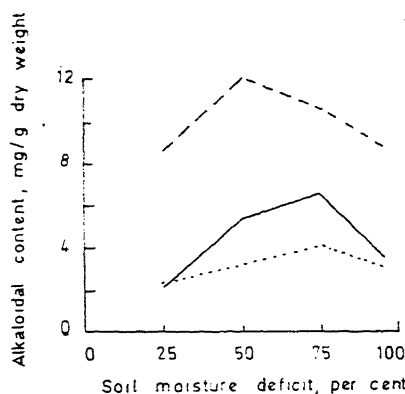


FIG. 1. Alkaloidal content as vincaleukoblastine in the leaves (---), stems (—) and roots (....) of *Catharanthus roseus* G. Don under the influence of soil moisture deficit.

TABLE II

Alkaloidal content (mg-dry weight) as vincaleukoblastine in the leaves, stems and roots of *Catharanthus roseus* G. Don under the influence of soil moisture deficit

| Soil moisture deficit | Leaves   | Stems   | Roots  |
|-----------------------|----------|---------|--------|
| 25                    | 309.4 b* | 155.8 c | 48.1 c |
| 50                    | 444.0 a  | 387.9 a | 64.0 a |
| 75                    | 329.7 b  | 366.1 b | 58.0 b |
| 95                    | 180.1 c  | 167.7 c | 28.8 d |

\* Means followed by the same letter within a column are insignificant difference at 5% level.

in Table II. From this table, it could be concluded that the highest alkaloidal contents as vincaleukoblastine in different plant organs were obtained at the level 50% followed by 75% SMD. On the other hand, at 95% SMD level, the leaves and roots contained the lowest alkaloidal content. In stems, this lower alkaloidal content was observed at both 25% and 95% SMD. It can be concluded that both leaves and stems could serve as the main organs of *Catharanthus roseus* G. Don for highest alkaloidal content as vincaleukoblastine.

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## LETTERS TO THE EDITOR

### OXIDATION OF REINECKE SALT WITH IODINE MONOCHLORIDE

REINECKE salt is widely employed for the determination of alkaloids and other medicinally important organic bases<sup>1</sup>. The oxidant iodine monochloride has been recently used for the determination of a variety of reductants<sup>2,3,4</sup>. It has now been found possible to use this oxidant for the determination of Reinecke salt.

as to keep the overall acidity at about 5 N. After keeping for 10 minutes, 20 ml 10% KI solution was added and the iodine liberated was titrated with standard thiosulphate solution to starch end-point. It may be noted that the determination of the unconsumed ICl is possible without separating the iodine already produced during oxidation; for details see reference 4 (a). Blanks were run concurrently; no blank correction was necessary.

TABLE I

*Oxidation of Reinecke salt with iodine monochloride*

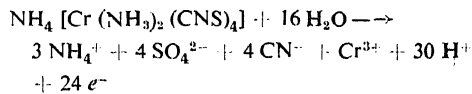
| Expt. No. | Reinecke salt taken (mmol) | ICl consumed (mmol) | Moles ICl consumed per mole of Reinecke salt | Reinecke salt found* (mmol) | % Error |
|-----------|----------------------------|---------------------|--|-----------------------------|---------|
| 1.        | 0.01675                    | 0.3997              | 23.86  | 0.01665                     | -0.60   |
| 2.        | 0.02260                    | 0.5438              | 24.06  | 0.02266                     | +0.27   |
| 3.        | 0.03350                    | 0.7995              | 23.86  | 0.03332                     | -0.54   |
| 4.        | 0.04519                    | 1.087               | 24.05  | 0.04529                     | +0.27   |
| 5.        | 0.05862                    | 1.404               | 23.95  | 0.05850                     | -0.22   |
| 6.        | 0.06699                    | 1.604               | 23.94  | 0.06683                     | -0.24   |

\* Assuming that 24 moles of oxidant are consumed per mole of Reinecke salt.

Standard aqueous solutions of Reinecke salt (Merck, proanalysis) were prepared and their strengths were checked by the chloramine-T method<sup>5</sup>. Stock solutions of iodine monochloride in 5 N HCl were prepared and standardised as described earlier<sup>3</sup>.

It was found necessary first to decompose Reinecke salt by warming it with sodium hydroxide before oxidation with iodine monochloride. Measured aliquots of the Reinecke salt solution were taken in 500 ml conical flasks and 5 ml 5 N sodium hydroxide solution was added. The flask was gently warmed for 10-15 minutes when the pink colour gradually faded to a dirty green. The system was cooled to room temperature and then the contents were added into a known excess of standard iodine monochloride solution taken in a stoppered conical flask; sufficient 10 N HCl was added to the oxidant so

Typical results are presented in Table I. It may be seen from the table that one mole of Reinecke salt consumes 24 moles of ICl, in accordance with the following reaction scheme:



It may be noted here that while in chloramine-T oxidation of Reinecke salt, oxidation of the CNS<sup>-</sup> proceeds upto CNO<sup>-</sup> stage it stops at the CN<sup>-</sup> stage in ICl oxidation, presumably owing to stabilisation of the powerful nucleophile CN<sup>-</sup> as ICN in the present system.

The author wishes to express his grateful thanks to Dr. C. G. R. Nair, Department of Chemistry,



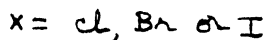
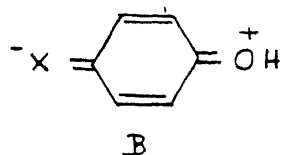
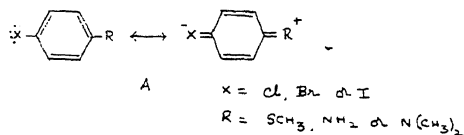
Kerala University, for his keen interest and helpful suggestions.

Chemistry Section, P. N. KRISHNAN NAMBIAN,  
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Poojapura, Trivandrum 695012, April 19, 1975.

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#### ADDITIONAL EVIDENCE FOR *d*-ORBITAL RESONANCE IN CHLORINE AND BROMINE FROM THE DISSOCIATION CONSTANTS OF PHENOLS IN THE EXCITED STATE

BALIAH<sup>1,2</sup> has shown that chlorine, bromine and iodine can under certain circumstances expand their valence shells by the utilization of their vacant *d*-orbitals. The importance of *d*-orbital resonance (such as A) will be significant when electron donating groups are present para to the halogen atom (Cl, Br or I).



Hazra and Lahiri<sup>3</sup> have recently reported the dissociation constants of some substituted phenols in the excited state. The dissociation constants of some phenols (pK, the ground state and pK\*, the excited state values) are given in Table I. In the ground state, *p*-fluorophenol is more acidic than phenol, but the other halogenophenols are much more acidic than phenol. These higher acidities have been explained by Baliah<sup>1</sup> on the basis that the resonance forms, such as B, make a significant

contribution to the structure of *p*-chloro-, *p*-bromo- and *p*-iodophenols due to the ability of Cl, Br or I to utilize their vacant *d*-orbitals.

TABLE I  
Dissociation constants of phenols in the ground and excited states<sup>†</sup>

|                         | pK                | pK*    |
|-------------------------|-------------------|--------|
| Phenol                  | 10.20             | 5.41   |
| <i>p</i> -Fluorophenol  | 9.90              | 5.50   |
| <i>p</i> -Chlorophenol  | 9.43              | 4.92   |
| <i>p</i> -Bromophenol   | 9.48              | 4.76   |
|                         | (9.36)            | (4.64) |
| <i>p</i> -Iodophenol    | 9.31 <sup>‡</sup> |        |
| <i>p</i> -Methoxyphenol | 10.20             | 6.22   |

<sup>†</sup> All the values are taken from reference 3 except those marked by ‡.

<sup>‡</sup> Value from reference 4.

In the excited state, all the phenols are more acidic and a careful examination of the pK\* values of the phenols listed in Table I reveals the following information: (i) The dissociation constant of *p*-fluorophenol is lower than phenol in the excited state and (ii) *p*-chlorophenol and *p*-bromophenol are more acidic than phenol, the acidities of the *p*-halogenophenols increasing in the order  $F < \text{Cl} < \text{Br}$ . Hazra and Lahiri<sup>3</sup> have not accounted for these observations. The higher acidities of *p*-chloro- and *p*-bromophenols, may now be attributed to the increased contributions of forms such as B in the excited state, since chlorine and bromine can act as electron acceptors and form *d* $\pi$ -bonding when present para to the electron releasing group, OH. Such a bonding is not possible with fluorine. The singular behaviour of fluorine in *p*-fluorophenol results by virtue of its + M effect; the fluorine atom in the para position opposes the electron release of the hydroxyl group. The dissociation constant of *p*-methoxyphenol is also lower in the excited state as expected. The pK\* values thus provide additional support for *d*-orbital resonance and *d*-orbital participation becomes significant in *p*-chloro- and *p*-bromophenols in the excited states.

The author wishes to express his sincere thanks to Professor V. Baliah for encouragement.

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COMPARATIVE TLC OF THREE LOCAL  
*OCIMUM* SPECIES

*Ocimum gratissimum* Linn., *O. basilicum* Linn. and *O. irvinei* J. K. Morton are the common *Ocimum* species found in and around Nsukka. *O. basilicum* (popularly known as curry), and *O. gratissimum* may be cultivated for culinary purposes. However, medicinal properties have also been ascribed to them (Oliver, 1960; El-Said *et al.*, 1969; Jain and Jain, 1973). *O. irvinei* grows wild as a weed and is not used as food. Information on it appears to be scanty. Although the leaves of these plants have perceptible difference in odour, presumably due to their essential oils, this investigation has been undertaken to determine how far these closely related plants can be distinguished chromatographically, taking advantage of the modality of some of their constituents.

## Materials and Methods

The leaves of the different species were gathered from healthy plants and dried in the laboratory. They were extracted with petroleum ether (b.p. 60°–80°), and the solutions chromatographed on silica gel plates with hexane. The plates were later exposed to iodine vapours. The spots given by leaf pigments were not taken into consideration in the evaluation of the results.

Some of the components revealed by iodine differentiation of the plants are shown on Table I.

TABLE I

| Plant                 | $R_f$ value of components |      |      |
|-----------------------|---------------------------|------|------|
|                       | 0.72                      | 0.84 | 0.90 |
| <i>O. gratissimum</i> | +                         | —    | +    |
| <i>O. basilicum</i>   | —                         | —    | +    |
| <i>O. irvinei</i>     | +                         | +    | +    |

Thus *O. gratissimum* did not give the component with  $R_f$  value 0.84; *O. basilicum* showed only the component with  $R_f$  value 0.90; whilst *O. irvinei* showed all the three components with  $R_f$  values 0.72, 0.84, and 0.90 respectively. The leaf pigments did not interfere since the green pigments remained at the base line, and the yellow pigments which migrated only attained the  $R_f$  value of 0.33.

Further work for the characterisation of the components of the isolated essential oil is in progress.

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INVOLVEMENT OF BIOTIN IN CAROTENE  
FORMATION BY *PHYCOMYCES BLAKES-*  
*LEEANUS* AND *BLAKESLEA TRISPORA*

CONSIDERABLE amounts of  $\beta$ -carotene have been reported to accumulate in various species of micro-organisms. The biosynthesis of carotenoids is very well worked out and forms the subject-matter of many recent reviews<sup>1-3</sup>. The first reaction in the biogenesis of  $\beta$ -carotene is the formation of acetoacetyl-CoA by the condensation of two molecules of acetyl-CoA. However, it is not known, whether the formation of acetoacetyl-CoA involves the biotin-dependant carboxylation reaction, yielding malonyl-CoA, as it is reported for fatty acid biogenesis in avian liver preparation<sup>4</sup> and in molds<sup>5-6</sup>. The present communication deals with the effect of addition of biotin and avidin on carotene formation in *Phycomyces blakesleeanus* and *Blakeslea trispora*.

The strain, medium composition and cultural conditions for *P. blakesleeanus* were same as described by Desai *et al.*<sup>7</sup>. The minus (—) (NRRL 2896) strain of *B. trispora* (obtained from U.S. Department of Agriculture, Peoria, Illinois) was maintained as described by Anderson *et al.*<sup>8</sup> and grown on a synthetic mucor medium (100 ml) described by Hesseltine and Anderson<sup>9</sup> at 30° C on a rotary shaker (150 r.p.m.).

Carotene was extracted and estimated as  $\beta$ -carotene as described earlier<sup>7</sup>. Lipid was extracted and estimated by the method of Folch *et al.*<sup>10</sup>. Biotin deficiency in these molds was created as described by Desai and Modi<sup>11</sup> by the addition of avidin (6 units, General Biochemicals, Ohio) in the culture medium for *P. blakesleeanus*, 50 ml and *B. trispora*, 100 ml. Biotin deficiency was confirmed by the microbiological assay, using *Lactobacillus arabinosus* as the test organism by the method of Skeggs<sup>12</sup>.

It was of interest to investigate the involvement of biotin in carotene formation in fungi. The results listed in Table I show that biotin stimulates the carotenogenesis with the concomitant increase in the lipid content of the molds. The results suggest the involvement of biotin in carotene formation in these

TABLE I

Effect of addition of biotin on carotene and lipid contents in normal and deficient cultures

| Biotin status<br>( $\mu\text{g}/\text{flask}$ ) | Total lipids<br>( $\text{mg}/\text{g}$ dry<br>cell weight) | Carotene ( $\mu\text{g}/\text{g}$ dry<br>cell weight) |           |
|---|--|---|-----------|
|   |  | Normal  | Deficient |
| <i>P. blakesleeanus</i>                         |  |   |           |
| Nil   | 120  | 327   | 109       |
| 50  | N.D.   | N.D.  | 205       |
| 100   | 270  | 421   | 313       |
| <i>B. trispora</i>                              |  |   |           |
| Nil   | 95   | 394   | 198       |
| 50  | N.D.   | N.D.  | 298       |
| 100   | 122  | 471   | 402       |

N.D. = Not determined.

The cultures of *P. blakesleeanus* and *B. trispora* were grown in a medium of 50 and 100 ml for a period of 10 days and 5 days, respectively. Deficiency of biotin was obtained by adding avidin 6 units per flask. Where mentioned specific amounts of biotin were added. All additions were done before inoculation.

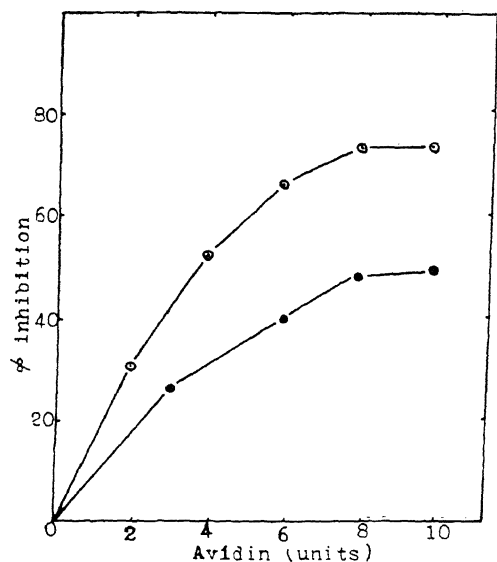


FIG. 1. Inhibition of carotenogenesis by avidin in *P. blakesleeanus* (○) and *B. trispora* (●).

cultures. Next, the effects of biotin deficiency on carotenogenesis was studied. The results in Fig. 1 show that biotin deficiency in these cultures caused about 50–70% inhibition of carotene formation. Further increase in the amount of avidin to the culture medium did not cause more inhibition in

carotene content. Addition of biotin to biotin-deficient cultures restored the carotene formation, to the level found in normal cultures (Table I) which further supported the involvement of biotin in some step during carotenogenesis. Similar results have been obtained in carotenogenic strain of *Neurospora crassa*, where omission of biotin from the medium resulted in complete inhibition of carotenogenesis (unpublished data). Biotin independent synthesis of acetoacetyl-CoA from acetyl-CoA has been reported by Bloomfield and Bloch<sup>13</sup>, Dakshinamurti and Desjardins<sup>14</sup> and Foster and Bloom<sup>15</sup>. Recently, *in vitro* studies of Neujahr and Bjork<sup>16</sup> demonstrate the involvement of both biotin-dependant and independent pathways for carotene synthesis in *B. trispora*. The data reported here suggest the partial involvement of biotin-dependant reactions for carotenogenesis in these molds.

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A NEW DISEASE OF GRAM  
(*CICER ARIETINUM* L.) IN INDIA

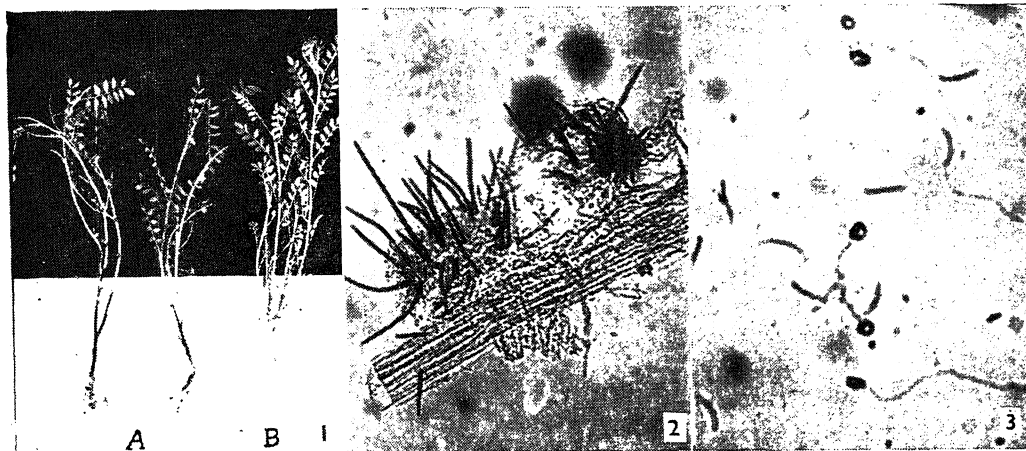
DURING the periodical survey of diseases of pulse crops, a severe disease of gram was observed at J.N. Krishi Vishwa Vidyalaya campus, Jabalpur and also at Indore, during October–November 1974. The affected varieties were local 'Kabuli' gram and G 62–404. Literature revealed that such a disease was not recorded earlier from India or elsewhere.

**Symptoms.**—All the aerial parts of gram plant were infected. The infected plants showed brown to dark brown discoloured sunken lesions at collar region near the soil surface. The necrotic lesion extended upto 6–7 cm from the soil level (Fig. 1). Generally the infection spread to the central branch which was completely girdled and invariably dead while other side branches rarely died although infected. In the severely affected plants, yellowing of lower leaves became conspicuous giving pale or sick appearance to the plants, and such devitalized plants were poor in vigour with arrested growth which sometimes died prematurely.

sterilized tap-water from a 7 days old culture in P.D.A. on 8 days old plants of a susceptible local 'Kabuli' gram. The inoculated plants were incubated in polythene bags for 5 days. Typical symptoms of the disease were incited on the seventh day. The diseased tissue yielded the same organism.

2. The plants were also grown on a sterilized paper fork dipped in water in test tubes and were inoculated by keeping 7 days old culture disks by the side of cotyledons of 8 days old gram plants of the same susceptible variety. Such inoculated plants were kept at 25° C for 7 days and symptoms were produced at collar region. Reisolations yielded the identical culture of the organism. Suitable controls maintained in both the sets of experiments remained healthy.

Abundant knob-like sclerotia (0.5 mm diam) were found in the culture. Conidia germinated readily in tap-water after 10–12 hours at room temperature (23±1° C) producing olive brown, round, clavate or irregularly thick walled appressoria at the end of germ tubes (Fig. 3). The acervuli and setae of



FIGS. 1–3. Fig. 1.(A) Affected plants showing symptoms; (B) Healthy plant. Fig. 2. Photomicrograph showing part of acervulus. Fig. 3. Photomicrograph showing germinating conidia and the formation of appressoria.

Temperature and high humidity favoured the disease development and spread during November but low temperature (December) was found to arrest the disease. The affected portion had numerous black bodies. Microscopic examination revealed them to be the typical acervuli (Fig. 2). In the carefully uprooted plants both epicotyle and hypocotyle regions of roots had dark brown to almost black discolorations. Single spore culture was obtained and the fungus was maintained on potato dextrose agar (P.D.A.) medium.

**Pathogenicity.**—1. Pathogenicity of the organism was established by spraying the spore suspension in

*Colletotrichum* are known to exhibit variation in size and are considered to be of little value in species differentiation<sup>1</sup>. The characters of conidia of the gram isolate are in agreement with those of *Colletotrichum dematium* (Fr.) Grove.

von Arx<sup>2</sup> on the basis of his extensive studies on the genus *Colletotrichum* has concluded that along with the characters of the conidia, the characters of appressoria and formation and nature of sclerotia, should also be considered important in species differentiation of *Colletotrichum*. In respect of these morphological characters the gram isolate resembles *C. dematium* (Pers ex Fr.) von Arx. The

causal organism is therefore identified as *C. dematium* as defined by von Arx<sup>2</sup> and Rajak<sup>3</sup>.

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#### A 'POLLEN VARIETY' OF *IMPATIENS* *ACAULIS* ARN. (BALSAMINACEAE)

*Impatiens acaulis* Arn. is a scapigerous balsam endemic to peninsular India. It is found abundantly during the monsoon season on the hilly slopes, especially near the water falls in the high ranges of Western Ghats. In 1836, Arnott<sup>1</sup> first described *I. acaulis* from the specimens collected in the Malabar region. The salient feature of the species is the 2-partite wings which distinguishes it from its near relative, *I. scapiflora* Heyne, wherein the wings are 3-lobed.

A detailed palynological analysis of *I. acaulis* and *I. scapiflora* from nearly 70 herbarium specimens (obtained from BLATT, BSI, CAL, MH and Herbarium of the Presidency College, Madras; grateful acknowledgement is made to the authorities of these herbaria for the loan of specimens) and the study of over 17 fresh specimens collected from various localities in the Western Ghats led to the discovery of a new kind of exine sculpturing in the genus<sup>2</sup>. During the present taxonomic treatment of the South Indian *Impatiens*, it was thought desirable to erect a variety of *I. acaulis* based on its distinct pollen characteristics.

The key based on pollen characters distinguishes the pollen variety as follows:

Pollen 4-colpate, tetrangular, exine simple, reticulate, muri simplibaculate.....

.....*I. acaulis* Arn. var. *acaulis*

Pollen 3-porate, radial, exine completely granulate.....*I. acaulis* Arn. var. *granulata*

*Impatiens acaulis* Arn. in Hook. *Comp. Bot. Mag.* 1: 325, 1836, var. *granulata* Bhask., Razi and Yog. var. nov.

Pollen varietas nomen *I. acaulis* Arn., var. *granulata* etsi alae bini lobus; pollen cum pori 3 et granulatus, hinc hic varietas abhorrens ab.

*I. acaulis* var. *acaulis* ubi pollen 4 colpus et reticulatum.

*Typus* lectus a Charmadi Ghat (Chickmagalur Dist.), alt. c. 1600 m., 26 Aug. 1972, V. Bhaskar 312 positus in Herb. Mysore University, Manasagangotri, Mysore. Paratype: *Yoganarasimhan* 1312 positus in Regional Research Centre, Bangalore.

Scapigerous erect herbs, tuberous; leaves radical, petiolate, 60–80 mm in length, ovate, cordate at base, margin crenate, pilose above, glabrous beneath, 5–7-nerved; inflorescence racemose, 20–25 cm in length, peduncle pink-tinged; flowers pedicellate, pedicel 25 mm in length, glabrous; bracts ovate-lanceolate; sepals ovate, apex rounded, 5-nerved (in Charmadi Ghat material) or 3-nerved and pigmented at apex (in Agumbe material); spur 2.5 cm long; wings distinctly 2-lobed, the larger anterior lobe fin-shaped, 20 mm in length, the smaller posterior lobe bent to a side, 13 mm in length, venation dichotomous, but veins connected to each other at margin of the lobes; fruits many-seeded, seeds minute, brown, hairy all over the surface, bands spiral.

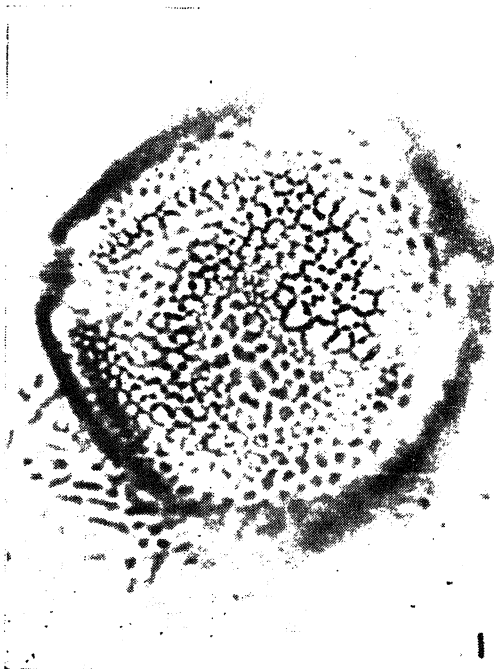
$n = 9, 10$ . Embryo sac monosporic, Polygonum type.

The description of the new variety is based on the fresh specimens collected by the senior author from Charmadi Ghat and Agumbe area. Herbarium specimens collected by W. A. Talbot from Karwar (N. Kanara) have also been examined. These, while showing the granulate condition of the exine, however, differ from the Charmadi specimens in having glabrous leaves, attenuate leaf bases and ovate, apiculate leaves thus resembling the specimens of *I. acaulis* var. *acaulis* from Udipi (S. Kanara). The new variety of *I. acaulis* is very abundant on rocky slopes, especially near water falls and on old bridges throughout the Charmadi Ghat and on rocks in Agumbe where water percolates during the monsoon. It is generally associated with other *Impatiens* spp., and species of *Begonia*, *Sonerila*, *Utricularia* and some blue-green algae.

Huynh<sup>3,4</sup> has studied the pollen morphology of nearly 350 species of *Impatiens* of the world including some 47 species from South India. He described the pollen morphology of *I. acaulis* as 3-colpate and reticulate (based on a study of Wight's 308 and 310). The present pollen sampling of this species has, however, revealed that *I. acaulis* is normally 4-colpate reticulate and simplibaculate in the different populations from South India (Fig. 1) and only the populations from Karwar, Charmadi and Agumbe exhibit granulate nature (Fig. 2). As to the new kind of exine sculpturing recorded here, Huynh (personal communication) after examining the material referred to him by the authors has stated, "I have surveyed your pollen preparation. It is quite true that the pollen grains have reached their

full maturity. The granulate sculpturing of the pollen of your plant is most interesting. I have never seen such a triaperturate pollen on my *Impatiens* material. In my opinion, the plant would be another species or a microform of it". In view of the fact that the scapigerous groups of species

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FIGS. 1-2. Fig. 1. Reticulate exine of *Impatiens acaulis* var. *acaulis* with simplibaculate muri ( $\times 3,000$ ). Fig. 2. Granulate exine of *I. acaulis* var. *granulata* var. nov. ( $\times 3,750$ ).

of *Impatiens* which are endemic to peninsular India are highly homogeneous and are distinguished hardly by one or two differences, and only the quantitative differences are very well pronounced rather than the qualitative features, it is felt that the granulate exine which is unique in the genus merits a varietal status for the taxon exhibiting it.

The authors are grateful to Dr. Kim-Lang Huynh for kindly examining the pollen material and offering his valuable opinion.

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#### **COLLETOTRICHUM GLOEOSPORIOIDES PENZIG —A MYCOPARASITE ON RAVENELIA SESSILIS BERKELEY**

A SURVEY of mycoparasites on the genus *Ravenelia* revealed parasitization of uredia and telia of *R. sessilis* Berk. on *Albizia lebbek* by *Colletotrichum gloeosporioides* Penzig. *R. sessilis* is common in Uttar Pradesh (Butler and Bisby<sup>1</sup>) and our collection was in the vicinity of Varanasi. The mycoparasite infected rust, pustules on the pods only, while sori on leaves remained uninfected. Only one other species of *Colletotrichum* parasitic on rust fungi has been reported earlier, viz., *C. urediniphilum* Hulea on *Aecidium muscardis* (Hulea<sup>2</sup>). Hence the present note, besides reporting a new rust mycoparasite, is also the first report of a *Colletotrichum* parasitizing *Ravenelia*. Observations on its isolation, pure culture, taxonomy and pathogenicity are presented here.

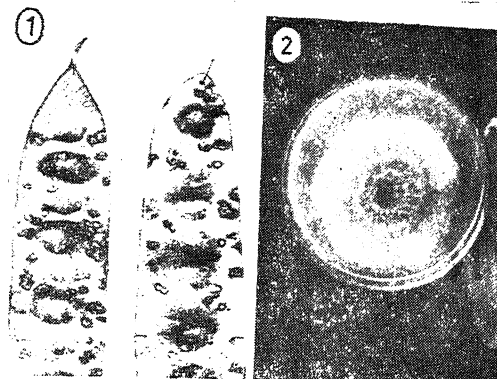
The pathogen was isolated from infected rust sori by the dilution plate method and maintained on

Potato Dextrose Agar. Isolation and pathogenicity tests were carried out on the uredia and telia developed on the pods as well as on leaflets suspended in 2% sucrose solution (Yarwood<sup>3</sup>). Spores of the pathogen, suspended in sterile distilled water, were sprayed with an atomizer on the uredia and telia and incubated for a week at room temperature ( $25 \pm 1^\circ \text{C}$ ). The pathogen developed as cushion-like pink acervuli along the periphery of the sorus, gradually converging towards the centre of the sorus, both on the pods and leaflets. Infection was confined to the rust sori alone and the surrounding tissues were unaffected, these symptoms being in conformity with the field infection on the pods. Reisolations from these yielded cultures similar to the original isolate in morphology and pathogenicity.

During October-November, whitish mycelial growth was repeatedly observed on uredia developing on the green mature pods but not on the leaflets. As the parasite grew and sporulated, it spread as salmon pink, diffuse mycelium on the rust sori, finally masking the whole sorus. The light brown uredia remain distinct enough, to differentiate the two infections. The mycoparasite flourishes in the uredia, until telia develop in the same sori replacing the uredia. Occasionally the mycelium infects the surrounding uredia, creeping over the pod surface. Early infection on the uredia partially reduces secondary uredial infection on the host parts. The salmon pink spots become studded with numerous minute acervuli. Infection becomes severe and profuse during November, completely masking the uredia on the pods (Fig. 1).

The fungus produces a dense cottony-white surface growth on PDA. The colour begins to change to ash grey, 4-5 days after inoculation, unlike in the natural infection. Minute cushion-like acervuli develop profusely around the point of inoculation and later extend towards the periphery. No rings or zones of acervuli are observed (Fig. 2). Repeated isolations on PDA produced similar cultures showing the absence of morphological variation. No sectoring in the colony is formed at any time, in any of the plated cultures. Mycelium is ash grey. A typical acervulus shows a stromatic mass of small, hyaline conidiophores bearing conidia apically. The conidia are hyaline, unicellular, smooth and thin walled, cylindrical with a few scattered vacuoles. Setose processes were not noticed in any of the culture isolates nor observed in field conditions. No ascigerous stage was developed in the culture or on the host part at any stage of development in the field or in the laboratory studies. Based on comparative observations on morphology and growth habits the pathogen is referred to as *Colletotrichum gloeosporioides* Penzig.

It differs, however, in pathogenic specificity and response to some major environmental factors and nutritional requirements from *C. gloeosporioides*. It is, therefore, proposed to accommodate this fungus as a new forma specialis of *C. gloeosporioides*. A formal description of the same is given below.



FIGS. 1-2. *Colletotrichum gloeosporioides* Penzig f. sp. *uredinicola* Singh on *Ravenelia sessilis* Berk. Fig. 1. Infection on uredia and telia on the pods of *Albizzia lebbek*,  $\times 1/4$ . Fig. 2. Plated culture on potato dextrose agar medium,  $\times 1/4$ .

*Colletotrichum gloeosporioides* Penzig f. sp. *uredinicola* Singh f. sp. nov.

Infection on uredia and telia on the host pods. Colonies salmon pink on the rust sori scattered on the pod surface, gradually spreading and finally masking the whole sorus, erumpent, cushion-like. Conidiophores hyaline and stumpy. Conidia hyaline, unicellular, cylindric to subcylindric, smooth and thin walled and measure  $11.0-18.8 \times 2.2-4.4 \mu$ . Setose processes absent.

On uredia and telia of *Ravenelia sessilis* Berk. on the pods of *Albizzia lebbek* Benth. at Varanasi, U.P., on November 15, 1964. Leg. U.P. Singh. TYPE.

A portion of the type material is being deposited in the herbarium of Commonwealth Mycological Institute, Kew, Surrey, England.

The author is grateful to Dr. J. A. von Arx for kindly confirming the identity of the organism.

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EFFECT OF DD, A NEMATOCIDE, ON SOME  
FUNGI *IN VITRO*

THE compound DD, which is a mixture of 1, 3-dichloropropene and 1,2-dichloropropane, is primarily a nematocide with limited fungicidal activity. As a soil fungicide it is used against *Phycomycetes*<sup>1</sup>. However, reports of its effectiveness in control of other soil-borne diseases have appeared from time to time. Venkata Ram<sup>2</sup> has found some control of root diseases of tea caused by *Fomes noxius*, *Rosellinia arcuata* and *Ustilina zonata* after treatment of soil with DD. More recently a combination of 1, 3-dichloropropene and chloropicrin was observed to delay infection of potato by *Verticillium albo-atrum*<sup>3</sup>. These observations prompted the present studies in which the effects of DD vapour on growth *in vitro*, respiration and (in one case) on sporulation of some fungi were investigated.

Nine cm Petri dishes containing potato dextrose agar medium inoculated in the centre with an 8 mm disc cut out from the margin of actively growing plate cultures of *Mucor* sp., *Ganoderma lucidum*, *Rhizoctonia solani* or *Fusarium javanicum* were exposed to different concentrations of DD in sterile garden soil, contained in suitable plastic containers following the method of Richardson and Munnecke<sup>4</sup>. Growth retardation was calculated from difference in colony diameter between control and treated cultures. Inoculum discs failing to grow were transferred to and incubated on fresh medium to distinguish between fungistatic and fungicidal effects. Dark grown plate cultures of *Cercospora personata* were placed under light for 12 hr to induce sporulation<sup>5</sup> and exposed to DD vapour as in other experiments in the dark for 12 hr and spore numbers determined. Oxygen uptake of test fungi was measured manometrically using discs cut out from periphery of cultures exposed to DD vapour for 6 hr. Results are presented in Table I.

*Ganoderma lucidum* and *R. solani* were inhibited by even the lower concentrations of the chemical. Only the highest concentration used showed perceptible effect on *Mucor* and *F. javanicum*. In the case of *Mucor*, the effect was only of a fungistatic nature since growth occurred on transfer of inoculum disc to a fresh medium. With the other three fungi there was a fungicidal effect at higher concentrations. Except in the case of *F. javanicum*, there was considerable inhibition of oxygen uptake with both concentrations of DD tried. Even in *F. javanicum* the higher concentration was quite inhibitory. Sporulation of *C. personata* was progressively inhibited by increas-

TABLE I

Effect of DD vapour on growth, O<sub>2</sub> uptake and sporulation of some fungi

| Test fungus                                  | Percentage inhibition of |      |                  |                       |      |      |
|--|--------------------------|------|------------------|-----------------------|------|------|
|  | growth/sporulation       |      |                  | O <sub>2</sub> uptake |      |      |
| <i>Effect on growth/O<sub>2</sub> uptake</i> | 0.1*                     | 0.2* | 0.4*             | 0.8*                  | 0.4* | 0.8* |
| <i>Mucor</i> sp.                             | 0                        | 0    | 0                | 100 <sup>a</sup>      | 64   | 81   |
| <i>Ganoderma lucidum</i>                     | 24                       | 56   | 100 <sup>b</sup> | 100 <sup>b</sup>      | 55   | 87   |
| <i>Rhizoctonia solani</i>                    | 27                       | 40   | 55               | 100 <sup>b</sup>      | 47   | ..   |
| <i>Fusarium javanicum</i>                    | 8                        | 10   | 22               | 100 <sup>b</sup>      | -24  | 80   |
| <i>Effect on sporulation</i>                 |                          |      |                  |                       |      |      |
| <i>Cercospora personata</i>                  | 33                       | 92   | 97               | 100                   |      |      |

\* Concentration of DD-ml/100 g soil.

<sup>a</sup> Fungus grew on transfer to fresh medium.<sup>b</sup> Fungus failed to grow on transfer to fresh medium.

ing concentrations of DD, the highest concentration giving complete inhibition.

The results suggest that DD can be effective against many fungi. Zentmyer and Kendrick<sup>6</sup> also found that besides *Phytophthora*, *Thielaviopsis* and *Rhizoctonia* too were susceptible to DD. In the present studies *Fusarium* and *Mucor* appear to be more resistant to DD than the other fungi. Resistance of *Fusarium* to DD has been observed by others also<sup>6-8</sup>. Respiration is affected by DD in all the fungi. The differences between the fungi in this regard might be a reflection of differences in permeability to DD vapour or due to other reasons. The inhibition of sporulation in *C. personata* will prove to be of interest should it turn out that the primary inoculum for infection of groundnut crop is spores produced on debris in soil. It should be possible to control several soil-borne diseases of fungal origin with suitable dosages and methods of application of DD.



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#### INITIATION OF PRIMARY THICKENING MERISTEM IN *DIOSCOREA GLABRA* ROXB.

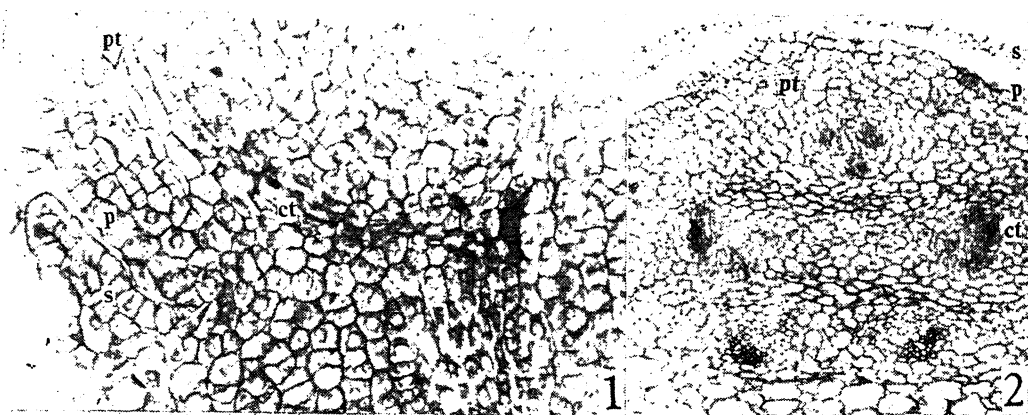
"CAMBIUM"<sup>1,2</sup>, the primary thickening meristem, is the key tissue in tuber genesis in *Dioscorea*<sup>1</sup>. According to Lawton and Lawton<sup>1</sup>, the meristem originates in the perivascular region (pericycle) of hypocotyl and, in some cases, seems continuous with the apical meristem of plumule. Sharma<sup>2</sup>, who studied the ontogeny of primary thickening meristem in connection with the origin and development of tuber of *D. glabra*, described it as arising in the perivascular ground tissue of hypocotyl. From the hypocotyl it extends into the rootstock and into the tubers—seedling and adult. To trace the

precise locus of origin of this meristem, the present study was made.

The seeds were sown in petri dishes on moist blotting paper at Palampur (alt. 1200 m) during June-July (June : max. temp. 29° C, min. temp. 23° C). They were sampled at the slightest emergence of radicle. Embryos were dissected out, and fixed in formalin-acetic acid-alcohol. Sections were stained in safranin and fast green, and mounted in D.P.X.

At resumption of growth of the embryo, the hypocotyl is just discernible. In transection it has four procambial strands of cotyledon traces—one (apparently double) belonging to the absorbent cotyledon and three to the emergent cotyledon (Fig. 2)—disposed in ground parenchyma, covered by epidermis. There are no procambial strands as yet to the plumular bud, which is represented by an undifferentiated mass of cells. The emergent cotyledon is quite small, just coming out of the sheath of absorbent cotyledon. The radicle end of hypocotyl is determined by the point of attachment of sheath of absorbent cotyledon below the insertion of emergent cotyledon (Fig. 1), and internally by the fusion of procambial strands of cotyledon traces, converging from opposite sides.

Directly below the emergent cotyledon, the ground parenchyma cells lying between the protoderm and the procambial strand of median cotyledon trace undergo periclinal divisions (Fig. 1). The resultant innermost derivatives initiate the primary thickening meristem (Figs. 1-2), which spreads in the perivascular parenchyma around the circumference of hypocotyl and to its radicle end, but does not extend into the primary root. The primary thickening meristem originates independent of the plumular apex, although later on it extends to the base of



FIGS. 1-2. Initiation of primary thickening meristem in sections of hypocotyl. Fig. 1. Vertical section,  $\times 280$ . Fig. 2. Transection,  $\times 100$ . (ct, cotyledon trace; p, protoderm; pt, primary thickening meristem; s, sheath of absorbent cotyledon.)

perennial bud produced in the axil of scale leaf (first leaf) of aerial shoot.

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### BLASTOMANIA—A NEW BUD TRANSMISSIBLE DISORDER OF CITRUS

THE survey carried out to screen the germplasm collection of citrus cultivars at the research station of All India Co-ordinated Fruits Improvement Project on citrus at Shrirampur, Maharashtra, during 1973 revealed the presence of one new disorder on Rangpur lime (*C. limonia*, Osbeck) growing on its own roots. The magnification was evident only on one branch. This note reports the symptomatology, results of transmission trials and the remission of symptoms with tetracycline.

The initial symptoms on foliage encompass drastically reduced leaf size, leaves forming a cluster-like boquet (Fig. 1). The clusters are chlorotic.



FIG. 1. Symptoms of blastomania disease on the branch of Rangpur lime. Note yellowing of foliage and reduction in leaf size.

The malformed leaves later drop down leaving the twigs either naked or with sparse foliage. The buds arising from leaf and twig axils give rise to multiple sprouts, these further developing into shoots and exhibiting the appearance typical to that of 'Witches broom'. The affected branch, which has short internodes and distorted twigs, subsequently dieback from apical end downwards. Formation of abortive flowers on some shoots was not uncommon.

The number of multiple shoots from a single bud varied from 8 to 11. This habit of excessive sprouting led the authors to designate this disorder as 'Blastomania' (Greek blastos = excessive development of buds; mania = madness).

Involvement of any insect vector was not encountered. Transmission of the disease was attempted by mechanical sap inoculation using 0.1 M phosphate buffer (pH 7.5) and 1 ml of sucrose solution plus 0.05 g of activated charcoal per gram of leaf material. The indicator plants used were *Chenopodium amaranticolor*, *C. quinoa*, *Gomphrona globosa*, *Vigna sinensis*, *Nicotiana glauca* and *Phaseolus vulgaris*. The efforts made in this direction failed. However, it was possible to transmit the disease with its typical symptoms in Rangpur lime (Fig. 2).



FIG. 2. The buddlings of Rangpur lime displaying multiple sprouting.

Initial suppression of the symptoms was noticed with 3 sprays of tetracycline hydrochloride given at fortnightly interval using a concentration of 500 ppm, suggesting that the disease is perhaps of mycoplasmic origin.

The disease blastomania resembles multiple sprouting virus disorder of sweet orange reported by Schwarz<sup>1</sup> from South Africa, in symptomatology. The disease was also transmitted mechanically by Schwarz<sup>1</sup> and Majorana and Schwarz<sup>2</sup>. Attempts are being made to find out the relation of blastomania with similar diseases.

From the results at hand, the disease appears to be of mycoplasmic origin and this is the first report from India. Further work to locate the MLO bodies in the phloem of infected tissues is in progress.

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#### PRESENCE OF ALKALINE PHOSPHATASE IN THE SEMINAL VESICLE OF *EUTYPHOEUS WALTONI* (MICH)

SEMINAL vesicle is a part of the male reproductive system of *Eutypoeus waltoni* and it is connected to the testis sac in the eleventh and twelfth segments. But in the adult worm, it extends backward and forward to occupy XI, XII, XIII and XIV segments. Its main function is to store excessive amount of seminal fluid.

The histology of seminal vesicle (Bhal, 1927) revealed an inner columnar epithelial cell layer which is surrounded by a double coat of muscle—the inner layer of longitudinal and the outer layer of circular muscle fibres, the circular coat, being more than twice the thickness of the longitudinal layer. The lumen of the sac is quite broad and the inner wall is produced into several diverticula which increase its capacity to store spermatid fluid.

The histochemical test employed to detect the presence of alkaline phosphatase included Gomori's Ca/Co, glycerophosphate technique (1952). The columnar cell layer showed large deposits of black precipitates of cobalt sulphide which roughly represented the sites of enzymatic activities of alkaline phosphatase. In longitudinal and circular muscle layer alkaline phosphatase was sparsely distributed. Controlled slide, prepared after eliminating sodium B glycerophosphate from the incubating mixture showed complete absence of black precipitate.

The present result demonstrating alkaline phosphatase activity in the columnar epithelial cells and longitudinal muscle, significantly points out that the enzyme is playing an important metabolic role at these sites. Alkaline phosphatase has been reported from the gonads of several insects (Nicola, 1938 b, 1949; Day, 1949; Wigglesworth 1965), in

nematodes (Jenkins, 1970; Jenkins and Erasmus, 1971) and in cestodes (Morris and Finneger, 1968; Bogitch, 1969; Arme, 1966 and etc.). These workers have suggested that alkaline phosphatase is concerned mainly with the active transport of chemical substances across the cell membrane. Further, Sharan (1971) has demonstrated that the presence of alkaline phosphatase in the prostate gland of *E. waltoni* is concerned with the dephosphorylation of phosphorylated hexoses and the resultant hexoses, are supposed to be stored in the gland to be used during adverse conditions.

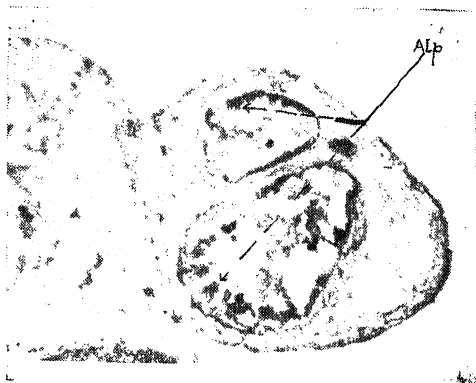


FIG. 1. Acetone fixed paraffin section of seminal vesicle showing alkaline phosphatase (ALP) in the columnar cells and longitudinal muscle,  $\times 100$ .

It may, therefore, be concluded from the above observations that alkaline phosphatase in seminal vesicle is involved in active transport of chemical substances across the cell membrane and also in the dephosphorylation of phosphorylated hexoses.

Thus from the additional sperm stored in the seminal vesicle, phosphorylated hexoses and other nutrients could be supposed to be absorbed by the columnar cells under the influence of alkaline phosphatase by means of active transport and gradually the entire additional sperm is metabolised.

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# PATH ANALYSIS OF SOME GROWTH PARAMETERS IN SORGHUMS

PATH analysis technique was first developed in crop by Wright (1921), for further analysis of correlation coefficients of dependent and independent variables. According to Dewey and Lu (1950) such an analysis together with regular correlation coefficients helps to visualise the importance of each yield attribute in a given crop variety. Several attempts have been made in this direction, taking mostly the grain yield components (Lal and Hague, 1971; Singh and Mehndiralla, 1970) as independent variables. Nevertheless, such a study is lacking on growth components and yield, despite the fact that the growth components are equally important (Krishnamurthy *et al.*, 1974).

The total photosynthate produced by any crop plant is generally dependent on both leaf area duration and net assimilation rate. This measures the total area available and the photosynthetic efficiency of leaves in providing dry matter to grain which is similar to NAR. With this background, relationship of these growth components, with grain yield in sorghum, has been worked out through path analysis.

Data involving both growth and yield analyses of several sorghum experiments conducted on red sandy loam soils at the PL-480 Project, Main Research Station, UAS, Bangalore, have been used for this study. The growth parameters, viz., Leaf area duration (D), NAR (Ea) and grain-leaf-ratio (G) were computed for all the experiments and correlated with grain yield. The authors in their earlier paper have already discussed the photosynthetic efficiency of Sorghum genotypes after heading (Krishnamurthy *et al.*, 1973). Here the relationship of the three growth parameters with grain yield have been studied following path analysis. The diagram (Fig. 1) shows the path analysis results of grain yield and growth components along with the direct correlation coefficients. It may be observed that while

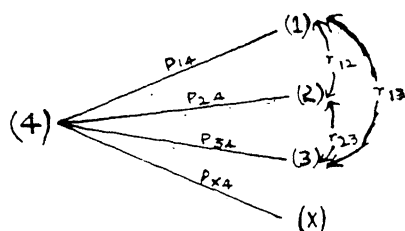


FIG. 1. Path diagram of growth components and yield. (1) Leaf area duration (D); (2) Grain-leaf ratio, (G); (3) Net assimilation rate (Ea); (4) Grain yield (Y); (X) Extraneous factors.

| Path coefficients |    | Correlation matrix |        |        |       |
|-------------------|----|--------------------|--------|--------|-------|
| $P_{14} = 0.4403$ |    | D                  | G      | Ea     | Y     |
| $P_{24} = 1.0020$ | D  | 1.000              | -0.367 | -0.550 | 0.020 |
| $P_{34} = 0.0957$ | G  |                    | 1.000  | 0.684  | 0.906 |
| $P_{44} = 0.1789$ | Ea |                    |        | 1.000  | 0.539 |
|                   | Y  |                    |        |        | 1.000 |

the direct correlation result obscured the relationship of yield with D, the path analysis showed a better relation. As regards the relationship of yield with G it was best as observed through both the correlation and path analysis results. The yield was highly correlated positively with 'G'. Contrary to this, the relationship of yield and NAR was low, as observed from both correlation and path analysis results.

This study further confirms the finding of Watson (1963) and Krishnamurthy *et al.* (1973), that (G) is relatively better related to yield than either NAR or LAD. Although the importance of LAD is by no means small, however in all growth analysis studies G will give a better picture of grain yield in genotypes.

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## CHROMOSOME NUMBER OF *ACRAEA VIOLAE* (LEPIDOPTERA : ACRAEIDAE)

In India the family Acraeidae is represented only by the genus *Acraea* with two species, viz., *A. issoria* (Hübner) and *A. violae* (Fabr.) : (Talbot<sup>1</sup>). The former is reported to occur only in North India; even though the latter was often considered to be a peninsular form, Wynter-Blyth<sup>2</sup> notes that it was earlier reported from North India also. The chromo-

some number of either of these species has not been reported.

The first record of the chromosome number in this genus is that of De Lesse and Condamin<sup>2</sup> who reported the haploid number of chromosomes as 32 in *A. bonasia* F. They<sup>4</sup> also reported 31 haploid chromosomes in *A. natalica pseudogina*. The present report of the chromosome number is the sixth in this genus.

Acetic-orcein squash preparations, without prefixation, of the testes of *A. violae* show 31 bivalents in metaphase I (Fig. 1). Little variation in size among the different bivalents was noted.



FIG. 1. Metaphase I chromosomes of *A. violae*.

The most common haploid chromosome number in Lepidoptera is 31 (Suomalainen<sup>5</sup>), although it is reported to vary from 8 to 191. The chromosome number of the genus *Acraea* seems to fall near the mean for the order.

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### EFFECTS OF SEED EXTRACT OF *LATHYRUS* ON THE SOMATIC CHROMOSOMES OF *MUS MUSCULUS*

A GOOD deal of work has been done on the effect of lathyrogens on different tissues of mammals<sup>1</sup>. Their effect on the hereditary vehicles of animals remains almost unexplored. However, an account on the cytological effects of the lathyrogen on somatic and germinal chromosomes of the plant and the animal has recently been reported<sup>2,3</sup>. Some neurotoxic substances and other chemical components have been isolated from the seeds of *Lathyrus sativus*<sup>4-10</sup> but their actual role in causing the disease is still a riddle. The present studies have been undertaken with a view to finding out the effects of the seed extract of *L. sativus* on the bone marrow chromosomes of the mouse, *Mus musculus*.

The seeds of *L. sativus* (25 g) were boiled with 100 ml distilled water for two hours. The supernatant fluid was cooled and filtered through No. 1 filter paper, and then injected intraperitoneally into mice weighing about 25 g at the rate of 0.25 ml per individual. The treated specimens were sacrificed after 4, 12, 24 and 48 hours respectively, after a short pre-treatment with 0.25 ml of 0.04% colchicine solution for 1½ hour. Control animal injected with identical volumes of distilled water were sacrificed after similar periods as in the case of the experimental series. The standard air drying method of preparing the bone marrow chromosomes was employed and the slides were stained in Giemsa using the phosphate buffer of a pH of 7.2<sup>11,12</sup>. Four specimens of either sex were used for each fixation period and fifty well spread metaphases were examined from each animal.

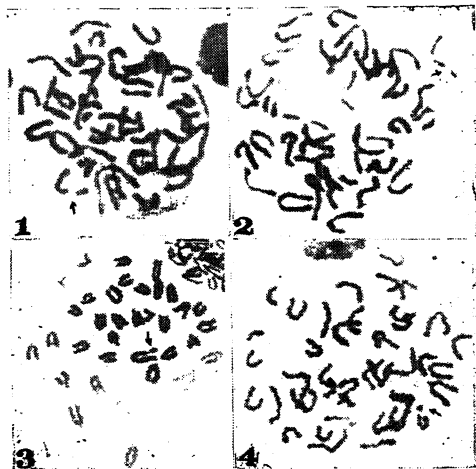
In the control series an examination of 800 metaphases from four fixation period revealed approximately 0.88% abnormalities in the form of fragments, gaps, achromatic lesions and chromatid constrictions (Table I). In the treated series, on the other hand, almost all common aberrations were noticed in the form of chromatid and sub-chromatid breaks (Figs. 1-3), chromatid constrictions, fragments of unknown origin (Fig. 4), gaps and achromatic lesions in all the time intervals. Apart from these common aberrations, some physiological effects, mainly in the form of loose spiralization of chromosomes were also noticed (Fig 4) in the cells of some mice. The details of the number and distribution of various types of aberrations in the treated series are given in Table I.

The occurrence of different aberrations in the treated series, as compared to those in the control series, indicates that the components, present in the seed extract of *L. sativus*, are responsible for the induction of chromosome aberrations in the

TABLE I

Frequency of different aberrations in control and treated series

| Series  | Hr. of Fixn. | Total No. of Metaphases | Aberration types and number |                             |                    |                 |                        | Total Aberrant |     |
|---------|--------------|-------------------------|-----------------------------|-----------------------------|--------------------|-----------------|------------------------|----------------|-----|
|         |              |                         | Chro-<br>matid<br>breaks    | Subchro-<br>matid<br>breaks | Constric-<br>tions | Frags-<br>ments | Gaps<br>and<br>Lesions | No.            | %   |
| Control | 4 Hr.        | 200                     | ..                          | ..                          | 2                  | ..              | 1                      | 3              | 1.5 |
|         | 12 Hr.       | 200                     | ..                          | ..                          | ..                 | 1               | ..                     | 1              | 0.5 |
|         | 24 Hr.       | 200                     | ..                          | ..                          | ..                 | ..              | 2                      | 2              | 1.0 |
|         | 48 Hr.       | 200                     | ..                          | ..                          | 1                  | ..              | ..                     | 1              | 0.5 |
| Treated | 4 Hr.        | 200                     | 4                           | 1                           | 4                  | 1               | 1                      | 11             | 5.5 |
|         | 12 Hr.       | 200                     | 2                           | 2                           | 3                  | ..              | 1                      | 8              | 4.0 |
|         | 24 Hr.       | 200                     | 3                           | ..                          | 1                  | 1               | 4                      | 9              | 4.5 |
|         | 48 Hr.       | 200                     | 1                           | ..                          | 3                  | ..              | 2                      | 6              | 3.0 |



Figs. 1-4

bone marrow cells of the mouse. However, the presence of a few chromosomal abnormalities in the control series could probably be due to certain automutagenic substances<sup>12-15</sup> or due to some altered physiological condition of the individuals<sup>16-19</sup>.

The seeds of *L. sativus* are known to contain L-homoarginine<sup>4,5</sup>, the neurotoxin  $\beta$ -N-Oxalyl-L- $\alpha$ - $\beta$ -diaminopropionic acid<sup>6</sup>,  $\beta$ -Oxalyl amino-L-alanine<sup>8</sup>,

N- $\beta$ -D-glycopyranosyl-N- $\alpha$ -L-arabinosyl- $\alpha$ - $\beta$ -diaminopropionitrile<sup>9</sup> and also a trypsin inhibitor<sup>10</sup>. The crude extract of the seeds of *L. sativus* said to contain 0.1 to 0.2% of different lathrogenic compounds<sup>2</sup>, are directly or indirectly responsible, for inducing chromosome aberrations in the somatic cells of mice.

The occurrence and continuation of break-type aberrations observed throughout the experiment indicate that the mutagenic substances acted on the chromosomes either in division or at G<sub>2</sub> phase of the cell cycle<sup>3-19</sup>. Moreover, the complete absence of chromosome type breaks, in the present experimental series, demonstrates that the chromosomes were not affected at 'S' phase or during 'D-D cycle'<sup>20</sup> of the cell division.

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#### ALARY POLYMORPHISM IN THE GALL THIRPS *BYCTOTHRIPS AYYARI* ANANTHAKRISHNAN (TUBULIFERA : INSECTA)

THE true gall forming nature of *Byctothrips ayyari* has now been confirmed through collections of a large series of the species from within hard leaf roll galls of *Memecylon lushingtoni* (Melastomaceae) from Coorg, South India, enabling a better understanding of the range of diversity of this species. The presence of a weak but distinct anteocular projection as well as a short subocular lateral prolongation are very characteristic of this genus, combined with the enlarged prothorax 1.4–1.5 times as long as head, the presence of two epimeral setae, absence of midlaterals, the armed foretibiae and strongly armed foretarsi. Though the earlier description was based only on a few brachypterous forms, significantly enough, the large series recently collected present a complete picture of alary polymorphism including in it apterous, brachypterous, hemimacropterous and macropterous individuals. The males are mostly apterous or brachypterous, rarely hemimacropterous, and micropterous individuals are unknown. Being much smaller than the females,

the range of variation among the males is very little and hence without oedymeres and gynaeccoids. A combination of alary polymorphism and allometric variation has so far been known to occur only on *Oncothrips tepperi* Moulton from Australia within galls of *Acacia oswaldii* (Mound, 1970)<sup>3</sup>. Micropterous and macropterous individuals with distinct structural diversities have hitherto been reported by Mound (1971)<sup>4</sup> in *Warithrips maelzeri* Mound and *Grypthrips mantis* Karny and to a limited extent in *Alocithrips hadrocerus* (Karny) (Ananthakrishnan, 1964<sup>1</sup>, 1969<sup>2</sup>). Among purely macropterous gall species such as those of *Arrhenothrips*, *Mesothrips*, *Gynaikothrips*, *Eothrips*, etc., structural diversities are often met with, resulting in distinct major and minor females. In *Byctothrips ayyari* the major females among the brachypterous as well as the macropterous forms do not show striking differences, but the overall differences between the minor and the major females, whether brachypterous or macropterous, are significant. As such we have in gall thrips in general (a) major and minor females showing distinct differences in form and accompanied by alary polymorphism, (b) major and minor females not associated with wing polymorphism, (c) no distinct major and minor forms, i.e., without significant structural diversity, with or without wing polymorphism. In cases where evident, only apterous and macropterous forms occur, without intermediates.

TABLE I

Showing analysis of wing polymorphs in *Byctothrips ayyari* Ananthakrishnan

| Types            | Wing length          | No. of individuals | Percentage of total No. |
|------------------|----------------------|--------------------|-------------------------|
| <b>Female :</b>  |                      |                    |                         |
| Wingless         | ..                   | 27                 | 20%                     |
| Brachypterous I  | 150–250              | 23                 | 17%                     |
| Brachypterous II | 250–300              | 49                 | 36%                     |
| Hemimacropterous | 350–450<br>(377–449) | 8                  | 6%                      |
| Macropterous     | 908–959              | 29                 | 21%                     |
| Total No.        |                      | 136                |                         |
| <b>Males :</b>   |                      |                    |                         |
| Wingless         | ..                   | 21                 | 46.5%                   |
| Brachypterous I  | 100–200              | 12                 | 27%                     |
| Brachypterous II | 200–316              | 9                  | 20%                     |
| Hemimacropterous | 370–377              | 2                  | 4.5%                    |
| Total No.        |                      | 44                 |                         |

Sex ratio: 7 : 2 Females : Males.

TABLE II  
Comparative morphometrics of *Byctothrips ayyari* showing wing polymorphism  
(in microns)

|                  |         | Head length/<br>width | Prothorax<br>length/width | Forefemoral<br>width | Pterothorax<br>length width | Foretibia<br>length | Foretarsal<br>tooth length |
|------------------|---------|-----------------------|---------------------------|----------------------|-----------------------------|---------------------|----------------------------|
| Wingless         | Females | 204-214               | 255-265                   | 234-255              | 306-357                     | 143-183             | 71-82                      |
|                  |         | 163-184               | 398-418                   | 153                  | 357-377                     |                     |                            |
|                  | Males   | 173-194               | 193-204                   | 153-194              | 275-316                     | 132-163             | 21-31                      |
|                  |         | 133-143               | 306-316                   | 92-102               | 296-306                     |                     |                            |
| Brachypterous I  | Females | 194-245               | 194-275                   | 194-235              | 235-367                     | 133-163             | 41-61                      |
|                  |         | 153-173               | 296-428                   | 102-153              | 296-326                     |                     |                            |
|                  | Males   | 173-183               | 184-214                   | 153-163              | 265                         | 112-122             | 31                         |
|                  |         | 153-163               | 316-347                   | 102                  | 316                         |                     |                            |
| Brachypterous II | Females | 214-224               | 244-265                   | 244-306              | 296-367                     | 133-143             | 61-82                      |
|                  |         | 173                   | 399-418                   | 133-173              | 347-377                     |                     |                            |
|                  | Males   | 163-204               | 173-194                   | 163-184              | 237-265                     | 112-122             | 31                         |
|                  |         | 143-153               | 316-326                   | 102                  | 296-316                     |                     |                            |
| Hemimacropterous |         | 194-204               | 265-275                   | 245-275              | 347-377                     | 153-173             | 71-82                      |
|                  |         | 173-184               | 398-428                   | 163-173              | 398-418                     |                     |                            |
| Macropterous     | Female  | 245-255               | 245-255                   | 235-245              | 337-357                     | 143-163             | 71-82                      |
|                  |         | 173-184               | 316-326                   | 122-132              | 388-408                     |                     |                            |

The impact of alary polymorphism in *Byctothrips ayyari* is reflected in the nature of eyes and ocelli, differences in the shape of antennal segments in some cases, the size of the subocular lateral prolongation, the size and shape of the forelegs and their armature, proportions of the thorax and the wide range of differences in the shape of the pelta (Fig. 1). The ratio of the head length/

counterparts it ranges from 1.2 to 1.4. The prothoracic length/width ratio in the apterous and brachypterous individuals approaches 0.6-0.9 and 0.7-0.8 in the macropterous forms. The forefemoral length/width ratio appears much greater in the macropterous forms (1.9), 1.8-1.9 in the hemimacropterous individuals and 1.5-1.9 in the apterous and brachypterous ones. Similarly the forefemoral/foretibial length ratio in the apterous and brachypterous forms is 1.4-1.6 and 1.5-1.6 in the macropterous. Table I provides an analysis of the wing polymorphs and Table II the comparative morphometrics of *Byctothrips ayyari* in relation to alary polymorphs.

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Entomology Research T. N. ANANTHAKRISHNAN.  
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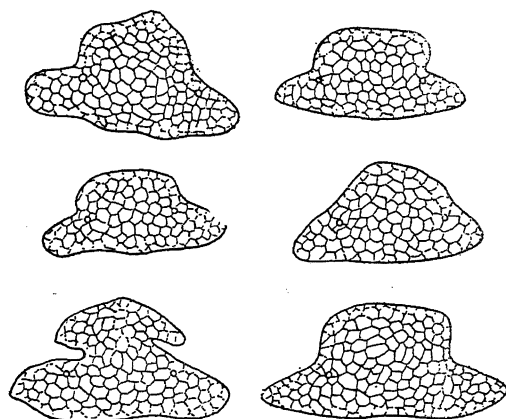


FIG. 1. Variations in the nature of the pelta. width is constant in the macropterous forms (1.4), while in the apterous and brachypterous

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**NUCLEAR POLYHEDROSIS OF  
*PLUSIA PEPONIS* F.  
(LEPIDOPTERA : NOCTUIDAE)**

LEPINE *et al.* (1953) reported the occurrence of nuclear polyhedrosis in *Plusia gamma* L. and Laudeho and Amargier (1963) recorded the incidence of polyhedrosis in *Plusia chaleytes* Esp. Nuclear polyhedrosis of *P. chaleytes* Esp. was also reported for the first time from India by Rabindra and Subramaniam (1975). Recently a group of larvae of *Plusia peponis* F. collected from gourds died of nuclear polyhedrosis in the laboratory. No previous report is available on the nuclear polyhedrosis of *P. peponis* F. and this seems to be the first record.

The virus collected and purified from diseased larvae in the laboratory was inoculated and tested in various instars of larvae of *P. peponis* F. Snake gourd leaves dipped in polyhedral suspension was fed to the caterpillars and mortality was recorded for different instars. The incubation period was found to be shorter in third and fourth instars with 4-6 days and 5-8 days respectively, compared with that of the fifth instar which had an incubation period of 7-10 days.

The polyhedral inclusion bodies in general were found to be roughly hexagonal and diameter of 50 polyhedra ranged from  $0.64\ \mu$  to  $2.32\ \mu$  with a mean of  $1.25\ \mu \pm 0.05$ . Electron micrograph of section of polyhedron (Fig. 1) showed virus rods to be occluded singly.

To study the histopathological changes due to polyhedrosis, fifth instar caterpillars inoculated with a dose of  $10^6$  polyhedra/larva were fixed in Alcoholic Bouin's fixative (Dubosque Brazil) at 24 hours interval and embedded in paraffin according to standard procedure. Sections  $6-8\ \mu$  were stained by a modified azan staining technique after Hamm (1966).

It was observed that the principal tissues affected were fat body, hypodermis, tracheal matrix and blood cells. Similar observations have been made by Drake and McEwen (1959) in the cabbage looper *Trichoplusia ni* (Hübner). Early signs of infection could be observed in fat body and hypodermis only 72 hours after inoculation and the symptoms of infection in the cells of the fat body was found to be a little more pronounced than those in the cells of hypodermis. Infection of tracheal matrix, gut epithelium, sarcolemma of muscle tissue, neurilemma of nerve tissue, and blood cells was noticed 96 hours after ingestion of virus. Though the nuclei in the cells of silk glands and malpighian tubules were found to be hypertrophied, no polyhedra could be observed in these tissues.

To find out the cross infectivity of the virus, 20 third instar larvae of the cabbage looper, *Trichoplusia ni* (Hübner) were fed with cabbage leaf treated with a heavy suspension of polyhedra isolated from *P. peponis* F. All the caterpillars died of polyhedrosis and the incubation period ranged from 6-8 days. The virus on inoculation to the original host *P. peponis* F. was found to be infective confirming the cross infectivity of the virus.

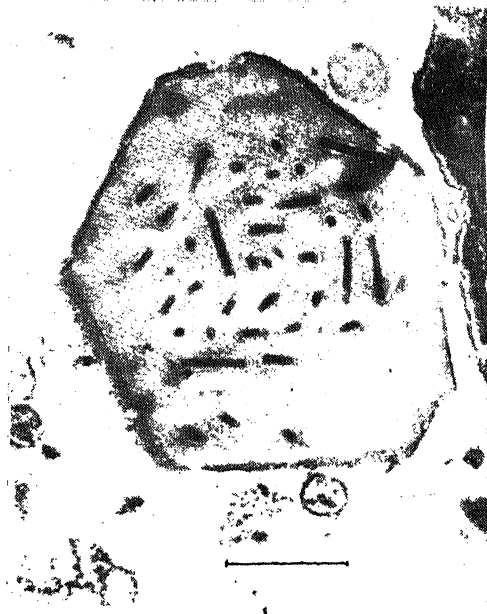


FIG. 1. Electron micrograph of section of polyhedron of *Plusia peponis* F. showing single embedded virions. Line =  $0.43\ \mu$ .

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**A NEW SPECIES OF TRICHOGRAMMATIDAE  
(HYMENOPTERA) REARED FROM THE EGGS  
OF OXYRACHIS TARANDUS FABR.  
(HOMOPTERA: MEMBRACIDAE)**

*Brachygrammatella* Girault

*Brachygrammatella* Girault, 1915, *Mem. Queensland Mus.*, 3 : 147.

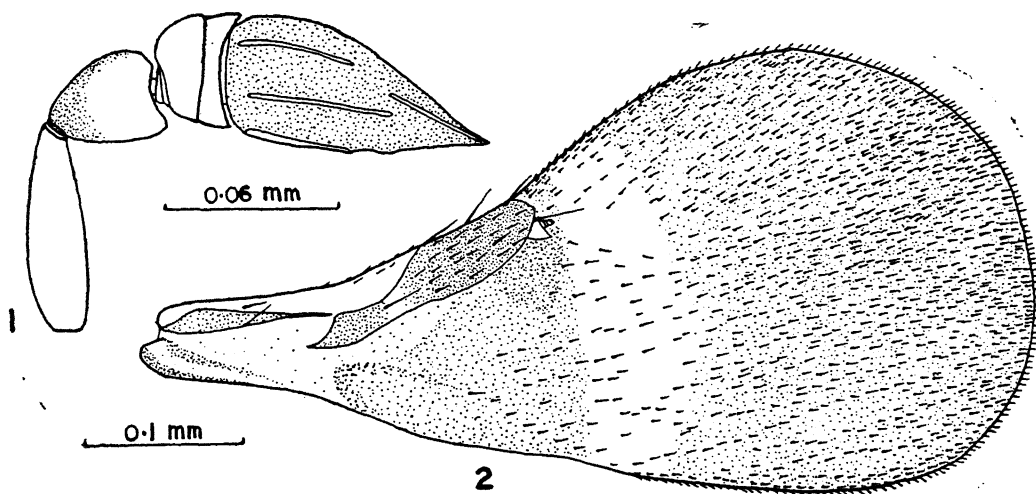
*Pseudbrachygramma* Girault, 1915, *Mem. Queensland Mus.*, 3 : 148.

Type species : *Brachygrammatella nebulosa* Girault.  
*Brachygrammatella longiclavata* sp. n. (Figs. 1, 2).

This species is more closely related to *Brachygrammatella perplexa* Girault, but differs from it in the following key characters :

1. For wings less than twice as long as wide ; marginal vein thrice as long as wide and with 16 setae ; stigmal vein well developed ; costal cell of fore wings short ; width of

facial view ; frontovertex wider than long ; ocelli red, arranged in obtuse triangle, basal ocellus separated by its own diameter from eye and occipital margins ; eyes red and smooth ; malar suture distinct ; antennae inserted at lower level of eyes ; mandibles tridentate with acute teeth. Antennae (Fig. 1) yellowish except basal portion of pedicel and club which are infuscated ; scape slightly more than three times as long as wide ; pedicel slightly longer than wide, longer than ring and funicle segments combined ; two indistinct ring segments present ; funicle 2-segmented, segments much wider than long ; club slightly more than twice as long as wide, much longer than pedicel and funicle combined, apex pointed. Thorax yellowish except two patches on mesoscutum, lateral sides of pronotum, metanotum, propodeum, pleurites and sternites which are infuscated ; pronotum constricted in middle ; parapsidal furrows complete. Fore wings (Fig. 2)



FIGS. 1-2. *Brachygrammatella longiclavata* sp. n. (1) Antenna, ♀ ; (2) Fore wing, ♀.

transverse hyaline band of fore wings more than the length of marginal vein.....  
.....*B. perplexa* Girault

- Fore wings twice as long as wide ; marginal vein less than two and a half times as long as wide and with 27 setae ; stigmal vein rudimentary ; costal cell of fore wings long and well developed ; width of transverse hyaline band of fore wings much less than the length of marginal vein  
.....*B. longiclavata* sp. n.

**Female.**

Head yellowish except genal and post-genal areas which are infuscated, longer than wide in

slightly infuscated with transverse hyaline band in middle, about twice as long as wide, broadly rounded at apex ; basal one-third naked ; submarginal vein long with an abrupt break in middle ; marginal vein dark brown, slightly more than twice as long as wide and with 27 setae ; stigmal vein rudimentary ; marginal fringe spaced by a distance equal to the length of setae. Hind wings hyaline, about five times as long as wide ; marginal fringe long, more than one-half of wing width, setae spaced by a distance equal to one-fifth their length. Legs dark brown except basal and apical portions of femora, basal and apical portions of tibiae and tarsi which are yellowish ; tarsi 3-segmented. Abdomen dark except base of dorsum which is yellow ; ovipositor concealed, arising from base of abdomen.

Female length : 0.75 mm.

Holotype ♀; 3 ♀ paratypes, India, Uttar Pradesh, Aligarh, Fort, ex eggs of *Oxyrachis tarandus* Fabr. on *Acacia* sp., 18-9-1974 (M. Younus Khan). Material in Zoological Museum, Aligarh Muslim University, Aligarh, India.

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# INHERITANCE OF GALL MIDGE (*PACHYDIPLOSI* *ORYZAE* WOOD MASON) RESISTANCE IN RICE WITH PARTICULAR REFERENCE TO CYTOPLASMIC INFLUENCE ON ITS EXPRESSION

Rice gall midge is a serious pest of rice. It has been reported that resistance to gall midge is controlled by 1 to 4 genes<sup>1,2,3,4</sup>. In order to throw further light on this phenomenon, studies were

carried out at the Central Rice Research Institute, with crosses amongst three resistant varieties, namely, W. 1263 (Eswarakora × MTU. 15), Shakti (PTB. 21 × PTB. 18) × IR. 8 and RPW. 6-13 IR. 8 × Siam. 29) and four susceptible varieties, CR. 129-118 (IR. 8 × LXZ-N) × IR. 8, IR. 20 (IR. 262 × TKM. 6), Ratna (TKM. 6 × IR. 8) and Vijaya (T<sub>0</sub>. 90 × IR. 8). The F<sub>1</sub> and F<sub>2</sub> plants were tested in the green house. In the case of F<sub>1</sub>, three plants from each cross were tested. Three to four weeks old plants were infested with fertilized eggs (having black spots) which were 40-60 hours old (Chatterji *et al.*, unpublished). This was repeated for the second time where visible symptom of silver shoot was not noticed. Silver shoots, fully or partly formed, were noticed within two weeks after infestation. Any plant showing silver shoot was classified as susceptible. The F<sub>2</sub> segregation data are presented in Table I.

The F<sub>1</sub>s of the first four crosses (Table I) showed resistant reaction suggesting the dominant nature of the gene controlling resistance. However, in the cross Vijaya × RPW. 6-13, the F<sub>1</sub> plants exhibited susceptible reaction although its reciprocal was resistant. The F<sub>1</sub> plants were resistant where RPW. 6-13 was utilised as female (Cross 4), whereas in the reciprocal (with Vijaya as female) the F<sub>1</sub> plants were susceptible. Further, out of 968 F<sub>2</sub> plants in the cross RPW. 6-13 × Vijaya, 702 plants exhibited resistant reaction giving a monogenic ratio (3 R : 1 S). In the case of Vijaya × RPW. 6-13, amongst the 662 F<sub>2</sub> plants, approximately 2% of the total population showed resistant reaction.

TABLE I  
Mode of segregation in F<sub>2</sub> generation

| Cross                  | Resistant        | Susceptible  | Total      | Ratio | $\chi^2$ | P-value   |
|------------------------|------------------|--------------|------------|-------|----------|-----------|
| 1. CR.129-118 × W.1263 | O=384<br>E=369   | 108<br>123   | 492<br>492 | 3 : 1 | 2.44     | 0.20-0.10 |
| 2. IR. 20 × Shakti     | O=404<br>E=427   | 120<br>131   | 524<br>524 | 3 : 1 | 1.31     | 0.30-0.20 |
| 3. Ratna × Shakti      | O=427<br>E=406.5 | 115<br>135.5 | 542<br>542 | 3 : 1 | 4.13     | 0.05-0.02 |
| 4. RPW. 6-13 × Vijaya  | O=702<br>E=726   | 266<br>242   | 968<br>968 | 3 : 1 | 3.17     | 0.05-0.10 |
| 5. Vijaya × RPW. 6-13  | O=13             | 649          | 662        |       |          |           |

O = Observed and E = Expected,

There was further segregation in  $F_3$  and the behaviour of the progenies is under study.

A 3(R) : 1(S) in the first three crosses showed that resistance was controlled by one dominant gene; however, a slight deviation in  $\chi^2$  was obtained in the cross Ratna  $\times$  Shakti (Table I). The present results support the findings of Venkatswamy (1973) and Satyanarayanaiah and Reddi (1974) who also obtained a monogenic ratio. The different ratios reported by other workers indicate that the resistant parents may differ in their genotypic constitution.

The differential reaction at the  $F_1$  stage in the cross RPW. 6-13  $\times$  Vijaya and its reciprocal suggesting cytoplasmic influence on the expression of resistance to gall midge is reported for the first time. This observation has practical significance from the point of view of breeding for gall midge resistance.

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#### MULTIPLICATION STUDIES OF *XANTHOMONAS ORYZAE* ISOLATES ON DIFFERENTIAL RICE VARIETIES\*

DUE to lack of effective chemical control measures, the use of resistant rice varieties such as BJ<sub>1</sub>, Waseaikoku and Sigadis, against bacterial leaf blight caused by *Xanthomonas oryzae*, has become common recently, in tropical Asia (AICRIP, 1969<sup>1</sup>; Ou *et al.*<sup>4</sup>, 1971; Kauffman and Rao<sup>3</sup>, 1972; Buddenhagen and Reddy<sup>2</sup>, 1972). Studies on the pathogen multiplication gives an idea about the mode and extent of infection and spread of the bacterium in resistant and susceptible host tissue, enabling us to understand better, the resistance mechanism and the type of host resistance involved. Reddy and Kauffman<sup>5</sup> (1973) studied multiplication and movement of the pathogen with a single isolate in resistant and

susceptible hosts. In the present investigation, the multiplication of two isolates in three differential rice varieties with varied host pathogen reaction types, i.e., compatible and incompatible host-isolate system, were studied.

#### Materials and Methods

The two isolates used in the present study are H 66 H 100 obtained from Gurdaspur and Ranaghat, respectively. BJ<sub>1</sub>, Waseaikoku and T(N)-1 were used as differential varieties. Population assays were made from the leaf discs removed after 0, 2, 4 and 6 days from inoculation (DAI), at the inoculation point, after 6, 8 and 10 DAI at 1 cm and 10, 12 and 14 DAI at 3 cm from inoculation point. This method was used to eliminate saprophytic contamination as the leaf tissue dried up in the susceptible varieties. From two inoculated leaves of each variety-isolate combinations, 5 mm discs were cut with a cork borer at the sites and at intervals already mentioned, surface sterilized in 95% alcohol for 10 seconds and transferred to a tube with 10 ml of sterile water. Each disc was crushed with a separate sterilized glass rod, allowed to set for 30 minutes and then mixed thoroughly with a supermixer. Dilutions were made with 1 ml sterile pipettes. The two highest dilutions were plated out by pipetting 0.1 ml of the suspension into PSA plates and spread with a sterile glass rod. They were incubated at 28° C for 48 hours and the single colonies that developed were counted.

#### Results

Lesion development was faster in the compatible host-pathogen systems (BJ<sub>1</sub>-H 100 and Waseaikoku-H 66) than in the incompatible one (BJ<sub>1</sub>-H 66 and Waseaikoku-H 100). The population trends of the two isolates on the three rice varieties varied, depending upon the leaf area assayed and on the genetically controlled host-pathogen interaction.

Population trends at the inoculation point were generally similar both in the compatible as well as incompatible host-pathogen system. In the BJ<sub>1</sub> leaf, for example, H 66 which was lowly virulent and a similar multiplication pattern to that of H 100, the isolate which was moderately virulent on BJ<sub>1</sub>. Population trends of both isolates started at about  $1 \times 10^5$  bacteria/leaf disc at 30 minutes and increased to 1000 times ( $1 \times 10^8$  bacteria/leaf disc) at 6 days after inoculation. The same trend was noticed for the isolate inoculated to Waseaikoku.

The population trends of the bacterium were markedly different at a distance of 1 cm below inoculation point as compared with those at inoculation point. In the compatible host-isolate system,

the bacterial population were much higher than that in the incompatible host-isolate systems when assayed at 6–10 days after inoculation. In T(N)-1, the population trends were similar for both the isolates, as the host-isolate system was compatible in both the cases (Fig. 1).

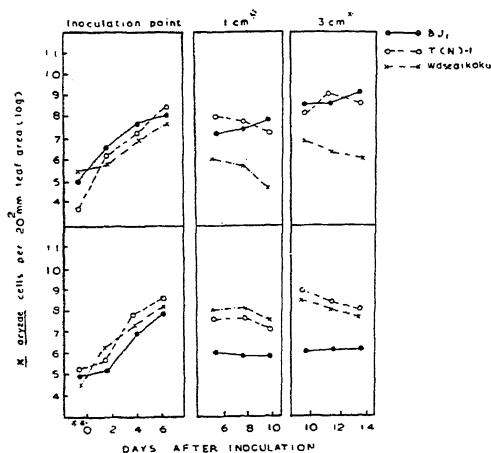


FIG. 1. Population trends of H 100 (Top row) and H 66 (Bottom row) isolates of *X. oryzae* in leaf tissues of BJ<sub>1</sub>, T(N)-1 and Waseaikoku at inoculation point, 1 and 3 cm below inoculation point of the inoculated leaves.

\* 1 and 3 cm below inoculation point.

\*\* 60 minutes after inoculation.

Each isolate had a remarkably similar population trend at the inoculation point in each of the three varieties. Population trends were also similar at 1 and 3 cm below the inoculation point for the compatible system.

A decline in the population of the bacterium was observed in Waseaikoku and T(N)-1 at 8 and 10 DAI at 1 cm and 12–14 DAI at 3 cm from inoculation point, which was probably due to the necrotic lesion advancing into these areas in many of the leaves. On BJ<sub>1</sub>, necrosis did not develop so rapidly.

#### Discussion

The population trends of the three differential isolates were similar on all varieties at the inoculation point. At 1 and 3 cm below the inoculation point, population trends were markedly different in the compatible and incompatible host-pathogen system. Same trend has been reported by Reddy and Kauffman<sup>5</sup> (1973).

Stall and Cook<sup>7</sup> (1966) and Scharen<sup>6</sup> (1959) observed equal multiplication of bacterium in general in both susceptible and resistant hosts. However, the population trends were lower in resistant hosts than those in susceptible ones, when

lower concentration of bacterium was inoculated. High population was inoculated to the leaves in the present study which may have accounted for similar population trends at inoculation point both in compatible and in incompatible host-pathogen systems. The leaf area assayed in this study was slightly larger than that done by Stall and Cook<sup>7</sup> (1966) but the bacterial populations at 0 time were very similar. The lower population trends of H 66 in BJ<sub>1</sub> and H 100 in Waseaikoku as compared to those of H 100 in BJ<sub>1</sub> and H 66 in Waseaikoku indicate that some sort of host specialisation exists in *Xanthomonas oryzae* indicating that distinct differences do exist in the genetically controlled ability of these two isolates to multiply and spread differentially in the respective varieties.

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#### ON FOLIAR SCLEREIDS IN A FEW SPECIES OF *SONNERATIA*

In recent years the utility of foliar sclereid typology in distinguishing species within the genus has been realised by many workers. With this objective in view, a few species of *Sonneratia* have been examined based on the reports of previously undescribed statements on foliar sclereids<sup>1-3</sup>.

*Sonneratia*.—*S. apetala* Buch.—Ham., Sundarbans, Wallich 3642 (CAL); *Pathuria* Sundarbans, Heing 54 (CAL); Burma, Pegu river, Kurz 1340 (CAL); Musadia and Baitarakud, Orissa Tidal Forest, L. K. Banerjee 8377 (CAL). *S. acida* L.f. syn. *S. caseolaris* (L) Engl., Sundarbans, Gamble 10085 (CAL); Anikhet Jungle Hill land, South Andaman,

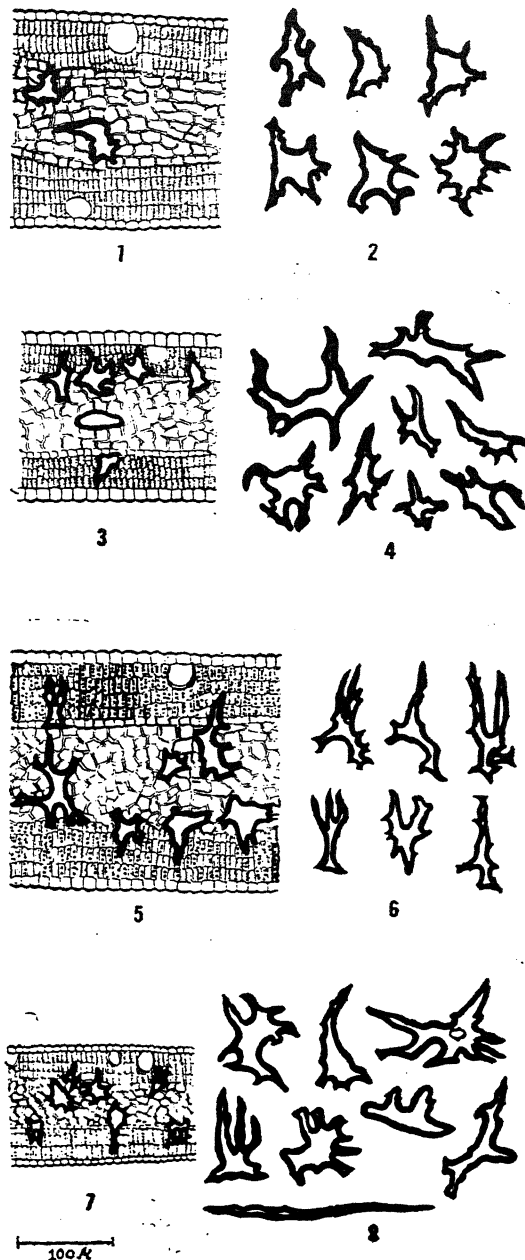
King s.n. (CAL) ; Akayal, Burma, Gilbert Rogers 155 (CAL) ; Orissa Tidal Forest, L. K. Banerjee 9445 (CAL). *S. alba* Sm., Pawut Island, Burma, Gilbert Rogers 427 M (CAL) ; Hukitola, Orissa Tidal Forest, L. K. Banerjee 8613 (CAL). *S. griffithii* Kurz., Straight Island, South Andaman, Prain 16 (CAL) ; Mergui, Burma, A. Meebold 14153 (CAL) ; Orissa Tidal Forest, L. K. Banerjee 10260 (CAL).

#### Methods

Leaves from the herbarium sheets of the foregoing species were partially cleared with 5% NaOH and then in supersaturated solution of chloral hydrate according to the technique of Foster<sup>4</sup>. The classification of Rao and Bhupal<sup>5</sup> is used for describing the various types of sclereids.

Cleared leaf expanses of the above-mentioned vouchered specimens of the four species of *Sonneratia* have revealed that diffuse sclereids are present in the mesophyll. In *S. apetala*, sclereids conform to vesiculose, rhizo and polyramous asymmetrical forms with innumerable irregular short or blunt processes (Fig. 2). The sclereids have, thick, striated cell wall and broad lumen of irregular width. In transections they are distributed in the aqueous tissue and very rarely, a few of their branches protrude into the adaxial and abaxial palisade tissues (Fig. 1). They form conspicuous idioblasts inside the aqueous tissue and do not show any relationship with vein-endings which, very often, inhibit dilated terminal tracheids. In *S. acida*, the sclereids conform to the types described under *S. apetala* (Fig. 4). However, there is a striking difference in their distribution inside the lamina. In transections, it is revealed that the sclereids have their main body inside the aqueous tissue and blunt drawn out processes directed towards the compact palisade tissue (Fig. 3). Structurally, they are similar to those of the sclereids of *S. apetala*. In *S. alba*, sclereids conform to rhizo and polyramous asymmetrical forms with blunt short or drawn out processes (Fig. 6). Structurally, sclereids have thick cell wall and lumina of irregular width. In transections, they are found to be distributed both in the palisade and the aqueous tissue (Fig. 5). They are densely distributed and sometimes it is not uncommon to observe their blunt processes showing sub-epidermal disposition. The vein-endings have well-developed terminal dilated tracheids and do not show any contact with sclereids. In *S. griffithii*, sclereids conform to rhizosclereids or fusiform to polyramous asymmetrical sclereids with short blunt processes (Fig. 8). Structurally, they have thick striated cell wall, pits and lumina of irregular width. In transections, mostly they are oriented in the

middle aqueous tissue, often with their processes directed towards the palisade layers (Fig. 7). In this respect there is a good deal of similarity with the sclereids of *S. acida*. However, the sclereids of *S. griffithii* are relatively bigger in size and densely packed in leaf expanse.



Figs. 1-8. Figs. 1-2. *S. apetala* ; Figs. 3-4. *S. acida* ; Figs. 5-6. *S. alba* ; Figs. 7-8. *S. griffithii*.

The current study has revealed that sclereids constitute a generic character in the genus *Sonneratia*. They are of diffuse pattern and do not show any relationship with terminal tracheids which are present at the vein-endings in all the investigated species. It is evident that sclereids of varied types are present within the mesophyll. Despite their variations, there is not much difference in the typology of sclereids within the genus from species to species. However, their distribution pattern inside the mesophyll is worthy of attention. Herein, there is a scope to utilise the internal patterning as a diagnostic character at the specific level. Accordingly, a key as an aid in the identification of species of *Sonneratia* has been built as follows :

#### Leaf Expanses

1. Sclereids mainly in the middle aqueous tissue : ..... *S. apetala*
2. Sclereids having their main body in the aqueous tissue with drawn out processes towards the adaxial and abaxial surface ..... *S. acida*
3. Sclereids sparsely distributed in the adaxial and abaxial palisade layers as well as in the middle aqueous tissue. .... *S. alba*
4. Sclereids densely distributed both in the palisade as well as in the middle aqueous tissue, sometimes towards the epidermal layers. .... *S. griffithii*

Thus the present study indicates that in the genus *Sonneratia* the typology of sclereids has limited diagnostic value and the sterile materials of this taxon could be identified only with the utmost exactitude in combination with their internal patterning.

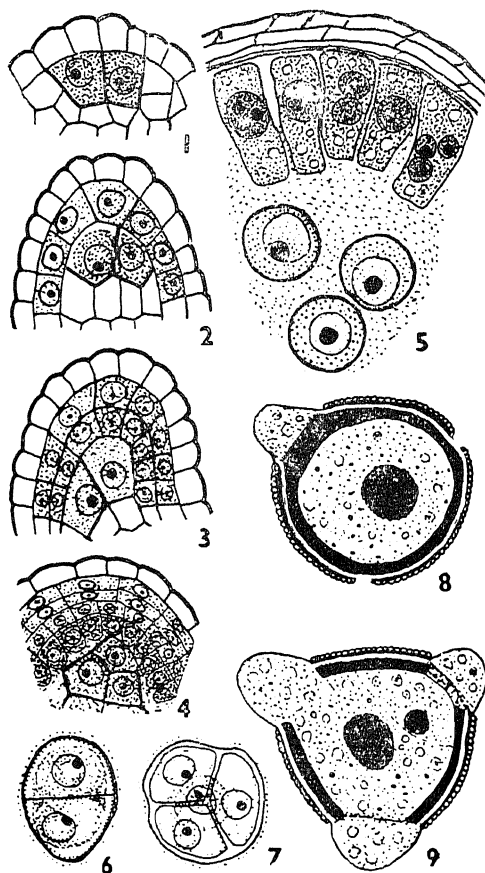
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#### ANTHER AND MALE GAMETOPHYTE DEVELOPMENT IN *TURNERA ULMIFOLIA* LINN. (VAR. *ANGUSTIFOLIA*, WILLD.)

*Turnera ulmifolia* is a West Indian plant with bright yellow flowers and lanceolate serrate leaves. It is a common weed of the family Turneraceae introduced to India (Gamble, 1967).

The anther is tetrasporangiate. In each locule, one or two archesporial cell(s) differentiate hypodermally and contain dense cytoplasm and conspicuous nuclei (Fig. 1). The archesporium divides to form the inner primary sporogenous cell(s) and an outer primary parietal cells(s) (Fig. 2). The latter by further periclinal division gives rise to two secondary parietal layers (Fig. 3). The outer secondary parietal layer, however, gives rise to two layers, the outermost differentiates into endothecium and the inner into a middle layer. The inner secondary parietal layer further divides and differentiates into a middle layer and tapetum (Fig. 4). The anther wall formation is of basic type



FIGS. 1-9. T.S. of anther. Fig. 1. Two archesporial cells. Figs. 2, 3. At early sporogenous cells stage; note the primary and secondary parietal layers, respectively. Fig. 4. At late sporogenous cells stage; note the differentiation of middle layers and tapetum. Fig. 5. Pollen mother cells. The tapetal cells have one to three nuclei. Figs. 6, 7. Dyad and tetrad stage, respectively. Figs. 8, 9. One and two-celled pollen, respectively. Incipient *in situ* germination is seen. (All figures,  $\times 500$ .)

(Davis, 1966). The maximum development of endothecium with fibrous thickenings is attained at the time when the pollen grains are about to shed. The middle layers are ephemeral and degenerate during meiosis (Fig. 5). The epidermis undergoes anticlinal divisions and become stretched, as the anther matures.

The tapetal cells are large with conspicuous nuclei and dense cytoplasm (Figs. 4, 5). During meiosis the tapetal cells undergo endomitotic divisions resulting in two to three nuclei in each cell (Fig. 5). In some cells two to nine nucleoli are seen. Towards the end of meiosis, the tapetal cells begin to lose contact with one another. The cells are highly vacuolated and degenerate at the mature pollen grains stage.

The primary sporogenous cells undergo a few mitotic divisions and finally differentiate into pollen mother cells (PMCs) (Figs. 2-5). The PMCs divide meiotically giving rise to dyads and tetrads (Figs. 6, 7). Microsporogenesis is of the simultaneous type and the arrangement of tetrads is tetrahedral (Fig. 7).

The pollen grain has a centrally situated nucleus (Fig. 8), it later divide itself into a small generative cell and large vegetative cell (Fig. 9). The mature pollen is shed at the two-celled stage of development. *In situ* pollen germination has been observed in all the anther locules (Fig. 9). The mature pollen grains are three colporate and the exine ornamentation is reticulate and intine is deeply stained (Fig. 9).

The author is grateful to Dr. P. S. Chikkannaiah for suggesting the problem, to Prof. M. S. Chennaveeraiah for facilities and to the Government of Karnataka for the financial assistance.

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#### EMBRYOLOGY OF TWO CULTIVARS OF *NERIUM INDICUM* MILL.

THE two cultivars of *Nerium indicum* Mill. differ greatly from each other in the structure of the flower, fruit and seed formation. In one, the flower consists of 5 petals and in the other the number of whorls and the number of petals in each whorl is variable. The former 'cultivar 1' produces

seeds and fruits and the later 'cultivar 2' is completely sterile and devoid of fruits and seeds. In view of these variations in the differential fertility and lack of detailed information on the embryology (except that of Andersson, 1931) of these cultivars, the present investigation is undertaken. Attention is paid to find out the causes of sterility in 'cultivar 2' on embryological grounds.

In the general structure and development of the anther, the two cultivars show essentially similar features, and hence, a common account is given. The anther is tetrasporangiate, and its wall consists of epidermis, sub-epidermal layer, 3 wall layers and a glandular tapetum (Fig. 1). The sub-epidermal layer later on develops into fibrous endothecium. Tapetal cells are two to three nucleate. The pollen mother cells undergo simultaneous cytokinesis and produce tetrahedral pollen tetrads. The pollen grains are triaperturate and shed at the 3-celled stage. The exine is smooth. Pollen polymorphism is observed. Three different sizes of pollen grains are recorded (Figs. 2-4). Some pollen grains are smaller measuring 12-15  $\mu$ , some are larger with 30-35  $\mu$ , and most of them are in the size range of 21-27  $\mu$ .

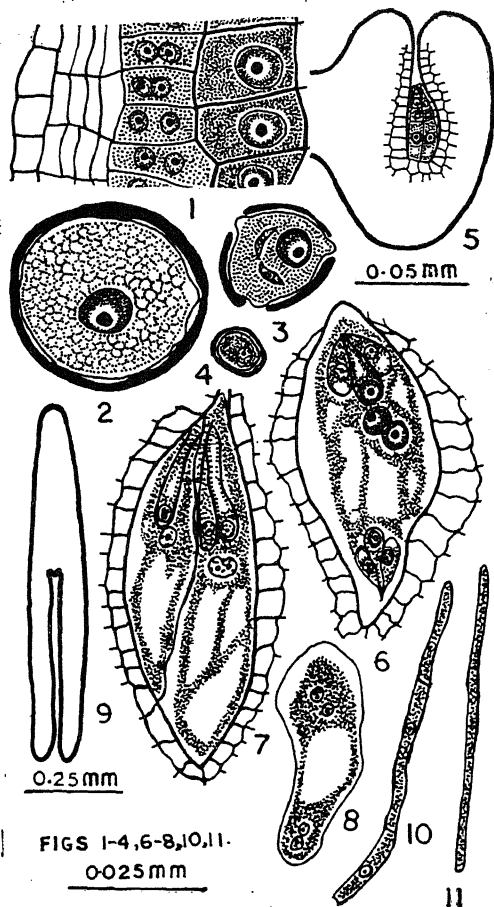
*In vitro* germination of pollen grains in different concentrations (i.e., 10, 5, 2 and 1%) of sucrose are conducted, but neither of the 2 cultivars showed satisfactory germination. But at higher concentration (25%) of sucrose, 'cultivar 1' showed some germination. Among these three sizes of pollen grains the percentage of germination is high in the medium sized ones. In a few cases dumb-bell-shaped pollen grains are noticed.

In both the cultivars the ovary is bicarpellary, syncarpous and bilocular with numerous ovules on axile placentation. The ovule is hemianatropous, unitegmic and tenuinucellate. In 'cultivar 1' the female archesporium is normally 1-celled and rarely 2-3 celled. Usually one and occasionally two or three archesporial cells develop into megaspore mother cells which undergo meiotic divisions and produce linear tetrads (Fig. 5). The chalazal megaspore develops into the 8-nucleate embryo sac of the Polygonum type (Fig. 6). Occurrence of double (Fig. 7) and triple embryo sacs is not uncommon. Fertilisation is porogamous. Syngamy and triple fusion occur almost simultaneously. Endosperm is *ab initio* Nuclear but later becomes Cellular. A well-developed embryo is formed (Fig. 9). Embryogeny conforms to the Caryophyllad type and is in agreement with the earlier observations of Mahlberg (1960). Polyembryony is rarely observed.

After fertilisation the epidermal cells of the integument towards sides become enlarged and accumulate deep brown contents. At about 4-8 celled stage of the embryo these cells divide and develop into



uniseriate multicellular hairs (Fig. 10). While the epidermal cells of the integument at the micropylar region elongate, accumulate abundant brown contents and directly develop into unicellular hairs (Fig. 11). These tufts of hairs from the micropylar region form a parachute mechanism and help in the dispersal of the seed. The fruit wall is massive and consists of parenchymatous cells. The epidermal cells of the fruit wall are large and distinct.



FIGS 1-4, 6-8, 10, 11.  
0.025 mm

FIGS. 1-11. *Nerium indicum* Mill. Fig. 1. L.s. part of anther lobe showing epidermis, wall layers, tapetum and pollen mother cells. Figs. 2-4. Pollen grains in different sizes. Fig. 5. L.s. Hemianatropous ovule showing 2 megaspore tetrads. Fig. 6. 8-nucleate embryo sac. Fig. 7. Double embryo sacs. Fig. 8. Degenerating 4-nucleate embryo sac of 'cultivar 2'. Fig. 9. Mature embryo. Figs. 10, 11. Multi and unicellular hairs of the seed respectively.

In the 'cultivar 2' ovarian degenerations are common. Although the development of the embryo sac

is of the Polygonum type as in 'cultivar 1' very rarely the embryo sac attains an 8-nucleate stage. Degenerations of embryo sacs at 2-4 nucleate stages is a frequent feature (Fig 8). Thus failure of germination of pollen grains, and degenerations of embryo sacs at 2-4 nucleate stage are the probable reasons for the failure of seed set in 'cultivar 2'. The work on the other factors for the failure of seed set in 'cultivar 2' are under investigation, and a detailed account can be published elsewhere.

We are thankful to late Prof. T. Sreeramulu, the then Head of the Department of Botany, Andhra University, Waltair, for facilities and encouragement. One of us (K. L. N.) is thankful to C.S.I.R. for the award of Junior Research Fellowship.

Department of Botany, H. MAHESWARI DEVI,  
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#### *OLPIDIOPSIS INDICA* SP. NOV. FROM INDIA

*Olpidiopsis indica* sp. nov., parasitizing *Pythium aphanidermatum* (Edson) Fitzpatrick, is characterized by the presence of one to three companion cells each with smooth walled resting spores.

During the course of study of lower fungi of Gorakhpur, a fallen decaying leaf of *Carica papaya* covered with fungal mycelium was collected from wet soil. When bacteria-free cultures of this fungal mycelium were grown on sterilized hemp-seed halves in sterile distilled water, the mycelium turned out to be that of *Pythium aphanidermatum* (Edson) Fitzpatrick. On subsequent examination some hyphae of this fungus showed the characteristic swellings caused by the parasite *Olpidiopsis*. Further, detailed observations have shown that this species of *Olpidiopsis* does not agree with any known species. The following description of this parasite is made from its infection in bacteria-free cultures of *Pythium aphanidermatum* growing on sterilized hemp-seed halves in sterile distilled water at 22-25° C.

*Olpidiopsis indica* sp. nov.

*Thallus* endobioticus, holocarpicus, efformans amplificationes sphaericas vel obovatus ad extremitatem hypharum hospitis vel in elementis lobulatis sporangiorum hospitis et generatim ducens ad hypharum septationem; zoosporangia solitaria vel multa, generatim ovata, sphaerica vel ellipsoidea, magnitudine variabilia, 17-27.4  $\mu$  diam. (13.6-35  $\times$  17-27.4  $\mu$ ) (quando ellipsoidea) pariete laeve, incolorata, tubis ejectionis 1-3, anguste cylindricis,

3.4–17  $\mu$  longis; *zoosporae* ovatae vel elongatae 3.5–5  $\mu$  longae, biflagellatae, flagellis fere aequalibus, prope apicem insertis; *sporaes quiescentes* semper unicus duabus vel tribus cellulis associatis, sphaericae, 13.6–28  $\mu$  diam., uno vel duobus globulis refringentibus; paries exosporae incolorata, laevis, 1.7–3.4  $\mu$  crassa; *cellulae associatae* ovatae vel aphericae, 6.4–10.5  $\mu$  diam., parietibus tenuibus, laevibus, incoloratis.

length, biflagellate, flagelliae almost equal in length, attached near the apical end; *resting spores* always with one, two or rarely three companion cells, spherical, 13.6–28  $\mu$  in diameter, with one or more refringent globules, exospore wall colourless, smooth, varying from 1.7 to 3.4  $\mu$  in thickness; *companion cells* oval or spherical, 6.8–10.5  $\mu$  in diameter, wall thin, smooth and colourless.

Parasitic in *Pythium aphanidermatum* (Edson) Fitzpatrick, collected from fallen decaying leaf of *Carica papaya*, Gorakhpur, U.P., India, October 9, 1973.

The host species has been identified with the help of key provided by Middleton (1943).

Living cultures of the fungus on the host are being maintained in the Mycological Laboratory, Department of Botany, St. Andrew's College, Gorakhpur, India (S-36). Preserved culture and two slides of the fungus are being deposited at the Indian Type Culture Collection, I.A.R.I., New Delhi.

#### Discussion

In an earlier communication the author (Srivastava, 1966) has pointed out that the two main characteristics considered together for the identification of the species of *Olpidiopsis* are: the identity of the host and the morphology of the exospore of the resting spore. The identity of *O. indica* is also based on these two characteristics.

Sparrow (1960, pp. 929 and 930) has listed four species of *Olpidiopsis*, *O. gracile*, *O. pythii*, *O. curvispinosa* and *O. brevispinosa*, parasitizing species of *Pythium*. These four species are placed under two categories: those having resting spores without companion cells and those bearing resting spores with companion cell. *Olpidiopsis gracile* and *O. pythii* have been placed under the first category, while *O. curvispinosa* and *O. brevispinosa* belong to the second category. Both the species bearing resting spores with companion cells are characterised by the presence of spines on the resting spore.

The present species also is characterised by the presence of companion cell with resting spore and therefore should be included in the second group of species of *Olpidiopsis* parasitizing *Pythium*. Out of the three species in this group, *O. indica* is the only one possessing smooth walled resting spores. Moreover, the number of companion cells attached with each resting spore in *O. indica* varies from 1 to 3 while in the other two species mentioned above only one companion cell is attached with each resting spore.

I wish to thank Prof. J. N. Couch of the University of North Carolina, for critically examining the manuscript. I also wish to thank Dr. C. J. Saldanha, Principal, St. Joseph's College, Bangalore, for the Latin diagnosis of the species, Dr. Y. B. Singh,

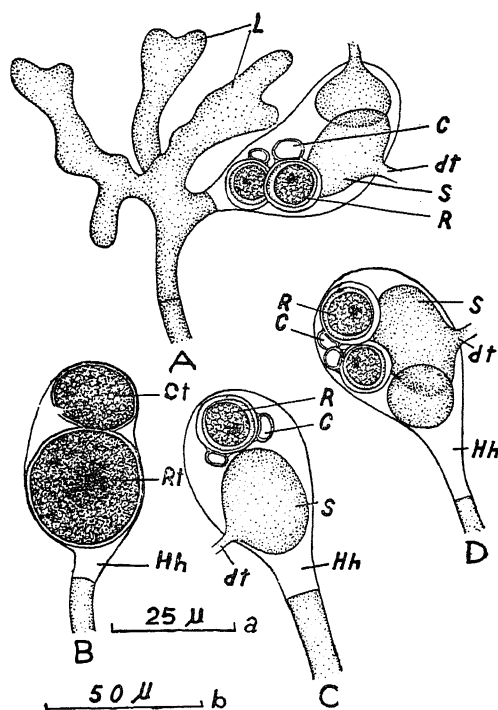


FIG. 1. A–D. *Olpidiopsis indica* (Camera lucida drawings). A. Two sporangia (S) with discharge tube (dt), resting spores (R) with companion cells (C) in lobulate elements (L) of host sporangia. B. Contributing thallus (Ct) and receptive thallus (Rt) in host hypha (Hh). C. Sporangium (S) and resting spore (R) with two companion cells (C) in balloon-shaped enlargement at the end of host hypha (Hh). D. Sporangia, resting spores and companion cells of the parasite in host hypha.

Fig. B. Scale a, the rest scale b.

*Thallus* endobiotic, holocarpic, causing oval, spherical or balloon-shaped enlargements at the end of the host hyphae or in lobulate elements of host sporangia, and usually leading to the septation of the hyphae; *zoosporangia* one to several, mostly oval, spherical or ellipsoidal, variable in size, 17–27.4  $\mu$  in diameter (13.6–35  $\times$  17–27.4  $\mu$  when ellipsoidal); wall smooth, colourless, discharge tube one to three, narrowly cylindrical, 3.4–17  $\mu$  in length; *zoospores* oval to elongated 3.5–5  $\mu$  in

Principal, St. Andrew's College, Gorakhpur, for providing facilities for the work and Mr. F. Abbasi for his help in the drawings.

Department of Botany, G. C. SRIVASTAVA.  
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### A NEW PLANT RECORD FOR INDIA— *BIDENS MINIMA* HUDS.

*Bidens minima* Huds (Astraceae) growing in wet places in the Dal Lake area of Srinagar is recorded for the first time from the Indian subcontinent. In Europe the taxon has been described as a variety of *Bidens cernua* L., *sen. lat.* *Bidens cernua* includes the plants with nodding heads as originally described by Linnaeus (1753). Hudson (1762) separated *B. minima* from *B. cernua* complex because of its small size, erect heads and smooth unequal spines of the achenes, and these characters seem to be constant in the local populations. As such the specific status for *B. minima* Hudson 1762 is maintained. The species has been described and illustrated for future reference.

*Bidens minima* Huds., FL. Ang. 310 (1762).

A dwarf marshy plant with few roots arising from the base of the stem. Stems simple, erect 2.5–8 cm high, cylindrical. Leaves oblong-lanceolate, opposite-decussate, 5–10 mm long, 1.5–2 mm broad, sessile, acute, entire with a single prominent mid-vein. Flower-heads terminal solitary (rarely 2), 5–7 mm diam., campanulate, erect; outer phyllaries 4–6, broadly lanceolate; inner 4–8, lanceolate or oblong, all yellowish with brown streaks; receptacle flat, naked. Florets homogamous few (9–15), 5 mm long, yellowish; stamens synanthrous, filaments flat, anthers compressed base hastate with an acute apical appendage; ovary tetragonal with 4 unequal bristles. Achenes 3–5 mm long, 1 mm broad, tetragonal, smooth, brown, with 2 short and 2 long, erect spines.

Flowers—September and Fruits—October.

Dal Lake; on floating islands.

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Srinagar, Kashmir, February 26, 1975.

### PRELIMINARY STUDIES ON THE GENETIC AND BREEDING BEHAVIOUR OF RADIATION INDUCED MACROMUTANTS IN BARLEY

THOUGH mutants are mostly deleterious, 'good' mutations have been found in barley. Mutation research in India has also resulted in the production of fully awned type in awnless New Pusha hexaploid wheats<sup>6</sup> and in the production of an awned mutant in an awnless paddy<sup>1</sup>. Verugheze and Swaminathan<sup>11</sup> also induced an amber colour mutant (Sarbat Sonora), in Sonora-64, a dwarf Mexican wheat, by use of X-rays and gamma rays. This paper describes four recessive gene mutations induced in barley by the application of gamma rays.

#### Material and Methods

Dry barley seeds of cultivar K<sub>12</sub> were irradiated with five gamma ray exposures of 10,000 R, 15,000 R, 20,000 R, 25,000 R and 30,000 R from cobalt<sup>60</sup> source at the Bhaba Atomic Research Centre, Trombay. 200 seeds from each treatment including the untreated normal were sown in the field for M<sub>1</sub>. Seeds from M<sub>1</sub> semi-sterile plants of each treatment were grown in M<sub>2</sub>. On the basis of morphogenetic differences, lethal, semi-lethal and viable mutants were classified into 18 distinct types.

Four mutants, namely, liguleless-auricleless (15,000 R); dense eared-triple awned (20,000 R), dwarf-erect (30,000 R) and cream-yellow-green (20,000 R) were selected and grown in M<sub>3</sub>. They were crossed with the normal parent and F<sub>1</sub> and F<sub>2</sub>

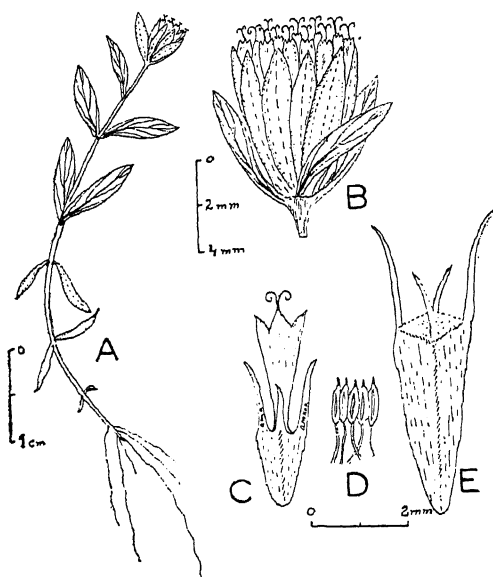


FIG. 1. *Bidens minima* Huds. A, Plant; B, Capitulum; C, Flower; D, Anther; E, Achene.

data collected for studying the inheritance of the mutant genes. Test of significance for the segregating population was made with the Chi-square ( $\chi^2$ ) method.

# Results

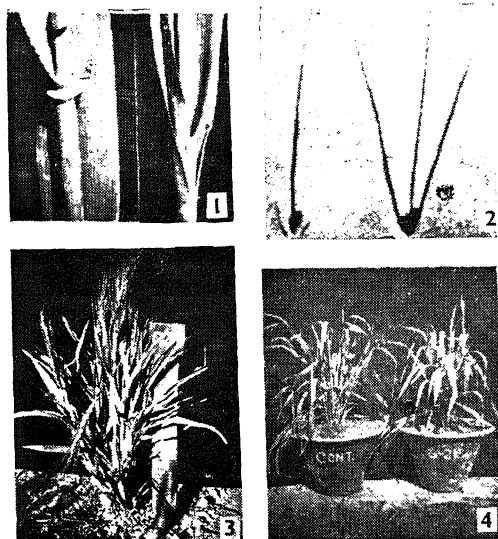
**Liguleless-auricleless mutant.**—The liguleless-auricleless mutant (Fig. 1) did not survive to maturity. This particular mutant is thus a homozygous lethal. The mutation was detected in the progeny of a translocation heterozygote, and every year a few mutant plants appear as lethal homozygotes in the progeny of such translocation heterozygotes. In one heterozygous line 17 normal plants and 7 liguleless-auricleless plants were obtained. For a 3:1 segregation,  $\chi^2 = 0.222$ ,  $P > 0.05$ . This is a single gene recessive mutation.

**Dwarf-erect mutant.**—This mutant showed very dwarf, erect and compact plants (Fig. 3). Leaves were short, broad and dark green. Spikes are short and compact with short awns. The lowermost internode of the rachis is elongated and has a tendency to droop. The mutant was crossed with the normal. The  $F_1$  produced normal plants, indicating that the dwarf character is recessive. But the Chi-square ( $\chi^2$ ) analysis could not be tried due to insufficient plants obtained in  $F_2$ .

**Cream-yellow-green mutant.**—The first three leaves of this mutant are pale green, while the subsequent leaves emerge as cream-yellow (Fig. 4). This chlorophyll deficient mutant was crossed with the standard normal. All  $F_1$  plants appeared normal green, and in  $F_2$ , 45 normal green, and 16 chlorophyll deficient plants were obtained. This fitted well to a 3:1 monohybrid ratio ( $\chi^2 = 0.245$ ,  $P > 0.05$ ). This character is thus due to a recessive gene mutation.

# Discussion

The above mutants have been reported earlier<sup>3</sup>. The liguleless-auricleless mutant segregated in a 3:1 ratio in  $F_2$  with no separation of the two characteristics. Schooler<sup>10</sup> considered that the liguleless-auricleless characters are completely linked.  $F_2$  data from the cross dense eared-triple awned mutant and normal revealed a monofactorial mode of inheritance. Several workers assigned the gene for elongated (awned) glume *e* to chromosome 2 of barley, while Doney<sup>2</sup> placed the gene for fine awned glume mutant in chromosome 1. Notz<sup>8</sup> and Nybom<sup>9</sup> induced about 10 similar mutations which were allelic to *E e* of the variety Triple-Bearded Mariout. Regular occurrence of a few liguleless-auricleless and dense eared-triple awned mutants in the progeny of their respective translocation heterozygotes suggests that the mutant genes for those two characters are located in one of the chromosomes involved in those two translocations at or near the break point (assuming that all translocation heterozygotes segregate and no non-translocation plants do). Earlier workers reported that most mutations that produce a dwarf phenotype, such as brachytic, many-noded dwarf and others, are recessive. The  $F_1$  of the cross between dwarf and normal produced normal plants, indicating that the dwarf character is recessive. The mutant also showed moderately elongated basal rachis internode. Similar X-ray induced mutations were reported by Kasha and Walker<sup>5</sup>. They concluded that the gene *lb*<sup>2</sup> was not allelic to *lb*, but *lb*<sup>8</sup> and *lb*<sup>m</sup> were allelic. It is not known whether the dwarf plant and the elongated basal rachis internode are two different mutations or two different



FIGS. 1-4. Fig. 1. Left: A normal tiller with ligule and auricle; right: liguleless and auricleless tiller. Fig. 2. Left: A normal spikelet with two small outer glumes; right: a mutant spikelet showing modification of the small outer glumes. Fig. 3. A dwarf erect mutant showing a drooping earhead. Fig. 4. Left: A normal green plant; right: a cream-yellow-green mutant.

**Dense eared-triple awned mutant.**—The mutant showed abnormal growth of the small outer glumes. The modified bases of the outer glumes and its enlarged tips appeared like two accessory awns (Fig. 2). This mutant plant was isolated from a translocation heterozygote. The mutant was crossed with the standard normal but grains were not formed. Offsprings of the original heterozygote segregated into 40 normal and 11 mutants. The  $\chi^2$  for 3:1 = 0.322,  $P > 0.05$ .

expressions of the same mutation. Of the above three *lb* mutations, *ib* and *ib*<sup>2</sup>, have been assigned to chromosome 1<sup>7</sup>. For dwarf mutations, one-, two-, and three-factor inheritance has been reported, but Heiner<sup>4</sup> concluded that such a character may be determined by a dominant gene. The F<sub>2</sub> data from the cross, cream-yellow-green and normal segregated in a 3:1 ratio, indicating that the mutant character is recessive.

Department of Botany,  
Gauhati University,  
Gauhati, Assam, March 3, 1975.

B. C. GOSWAMI.

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- \* Original not seen.

## SHORT SCIENTIFIC NOTES

### Some New Records of Snakes (Reptilia : Serpentes) from Jammu and Kashmir State

During our extensive investigations on the faunistic survey of the herpetiles inhabiting Jammu and Kashmir State, the following species of snakes were recorded from the type localities shown against

each. This, however, adds to the bulk of already known ophidian fauna of the State.

Eighteen species of snakes which are documented in this paper belong to five families and fourteen genera.

| Sl. No.                    | Snake species                               | Type locality   |
|----------------------------|---|---|
| Family: <i>TYPHLOPIDAE</i> |   |   |
| 1.                         | <i>Typhlops porrectus</i> Stoliczka         | .. Reasi, Udhampur, Kathua and Bhaderwah.   |
| 2.                         | <i>Typhlops braminus</i> (Daudin)           | .. Jammu and Udhampur.  |
| Family: <i>BOIDAE</i>      |   |   |
| 3.                         | <i>Eryx conicus</i> (Schneider)             | .. Chhamb Sector (Jammu), Kathua, Udhampur and Bhaderwah.                           |
| 4.                         | <i>Eryx johni</i> (Russel)                  | .. Bhaderwah, Kathua, Jammu and Udhampur.   |
| Family: <i>COLUBRIDAE</i>  |   |   |
| 5.                         | <i>Boiga trigonata</i> (Schneider)          | .. Bhaderwah, Nagrota (Jammu), Udhampur and Baramullah.                             |
| 6.                         | <i>Boiga multifasciatus</i> (Blyth)         | .. Udhampur, Jammu, Srinagar, Bhaderwah.  |
| 7.                         | <i>Coluber rhodorachis</i> (Jan)            | .. Udhampur, Bhaderwah and Srinagar.  |
| 8.                         | <i>Ptyas mucosus</i> * (Linn.)              | .. Reasi, Kathua, Udhampur, Ramnagar (Jammu), Bhaderwah, Uri and Anantnag (Kashmir) |
| 9.                         | <i>Sphalerosophis articeps</i> (Fisher)     | .. Udhampur Kathua and Bhaderwah.   |
| 10.                        | <i>Sphalerosophis arenarius</i> (Boulenger) | .. Jammu and Bhaderwah.   |
| 11.                        | <i>Trachischium fuscum</i> (Blyth)          | .. Kathua.  |
| 12.                        | <i>Amphiesma stolata</i> ** (Linn.)         | .. Anantnag (Srinagar).   |
| 13.                        | <i>Lycodon travancoricus</i> (Beddome)      | .. Jammu, Udhampur and Bhaderwah.   |
| 14.                        | <i>Fowlea piscator</i> (Schneider)          | .. Udhampur and Bhaderwah.  |
| Family: <i>ELAPIDAE</i>    |   |   |
| 15.                        | <i>Bungurus coerulus</i> (Schneider)        | .. Udhampur, Kathua, Jammu and Bhaderwah.   |
| 16.                        | <i>Naja naja oxiana</i> (Eichwald)          | .. Kathua, Chhamb and Udhampur.   |
| Family: <i>VIPERIDAE</i>   |   |   |
| 17.                        | <i>Echis carinatus</i> (Schneider)          | .. Jammu, Kathua and Udhampur.  |
| 18.                        | <i>Vipera russeli</i> (Shaw)                | .. Jammu, Kathua, Udhampur and Bhaderwah.   |

\* Common in J and K State.

\*\* Common in Jammu region.

Our thanks are due to Shri T. S. N. Murthy, of Southern Regional Station, Zoological Survey of India, for his sincere help in the positive determination of the species of snakes. One of us (BDS) is thankful to the Principal, Government College, Poonch, for his constant encouragement.

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### Triticale Mutants with Amber Coloured Seeds

Proper seed development with attractive colour of the seed coat is a major problem with the existing varieties of triticales. Efforts to solve this problem through pedigree and mutation breeding is in progress at Genetics Division, I.A.R.I. An encouraging result obtained through mutation breeding is reported here.

Seeds of triticales strain S. 141 developed here were treated with aqueous solution of nitrosomethyl urea (NMU). The seeds were soaked in distilled water for 16 hours. Then they were transferred into 0.01% of NMU for 6 hours. Then the seeds were thoroughly washed with water and sown in field to raise the M<sup>1</sup> generation, in 1971-72 rabi. M<sub>2</sub> population of about 20,000 plants derived from selected seeds of 500 M<sub>1</sub> plants was critically screened for seed colour variation. Two plants could be identified which had attractive amber colour and better developed seeds. Isolation of such types marks a significant step in triticales breeding. This forms the first report on progressive mutants of triticales with amber and better filled grain.

Table I gives the comparative idea of the mutants and the control.

TABLE I  
Comparison of triticales mutants with the control

| Character         | Control<br>(S-141) | Mutant-I | Mutant-II |
|-------------------|--------------------|----------|-----------|
| Plant height (cm) | 132.50             | 125.00   | 130.00    |
| Days to flowering | 97                 | 93       | 92        |
| Days to maturity  | 159                | 149      | 157       |
| Seeds/Spike       | 72                 | 86       | 80        |
| Fertility (as %)  | 61                 | 75       | 72        |
| Seed coat colour  | Brown              | Amber    | Amber     |
| Seed protein %    | 14.2               | 17.8     | 17.2      |

Stabilization and evaluation of these mutants is in progress. These are also being used in triticales improvement programme.

Division of Genetics, V. RAMANATHA RAO.  
Indian Agricultural M. G. JOSHI.  
Research Institute,  
New Delhi-12, April 7, 1975.

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### ANNOUNCEMENTS

#### The Mehta Research Institute of Mathematics and Mathematical Physics

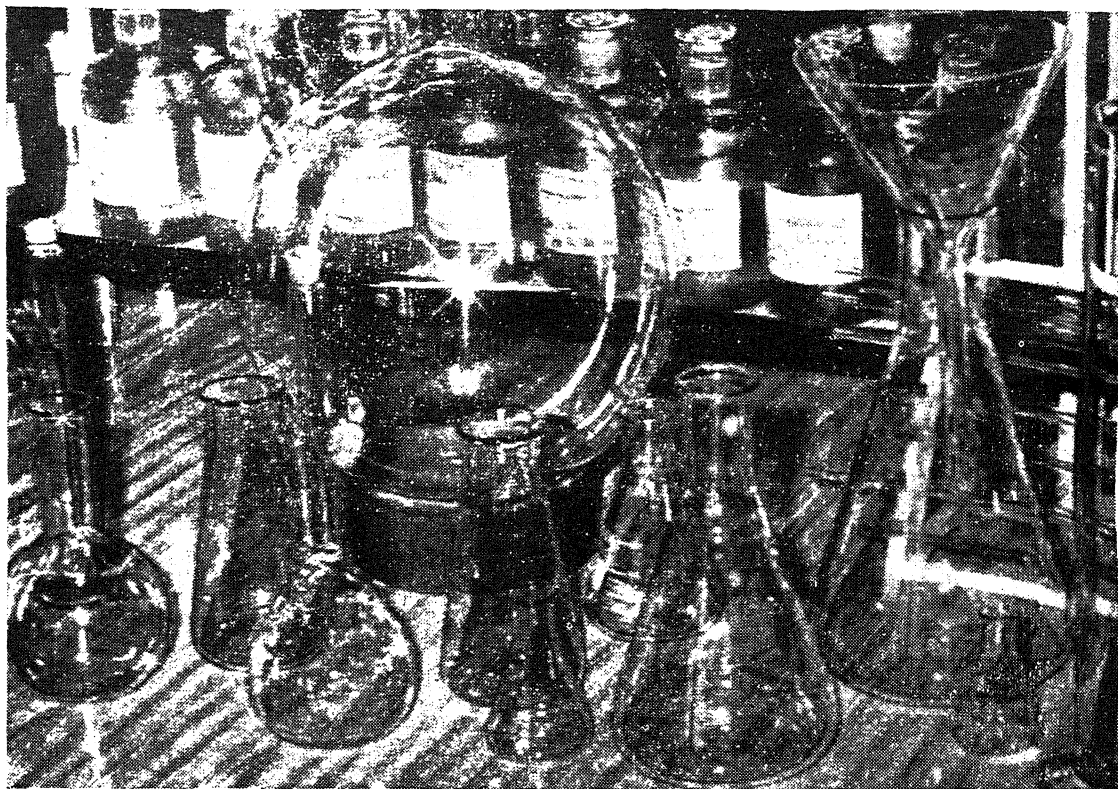
With the financial help of Mehta Trust and the grants from the Governments of India and Uttar Pradesh the above Research Institute has been started at 26, Dilkusha, New Katra, Allahabad-2. In the first phase (1975-78) the Institute will devote itself to the following branches of Mathematics :

- (1) *Pure Mathematics* :
  - (a) Mathematical Analysis,
  - (b) Functional Analysis,
  - (c) Theory and Methods of Solution of Ordinary and Partial Differential Equations, etc.
- (2) *Applications of Mathematics* :
  - (a) Environmental Dynamics,
  - (b) Probability Theory, Stochastic Processes, Information Theory,
  - (c) Mathematical Education,
  - (d) Mathematical Models and Techniques in Educational Systems, Evaluation, etc.

3. During the second phase research facilities will be developed in the following branches :

- (a) Non-equilibrium Thermodynamics,
- (b) Quantum Physics, Phase Transitions,
- (c) Relativistic Mechanics, General Relativity.

4. Prof. P. L. Bhatnagar, who is known for his contributions to Applied Mathematics, has taken charge of the Institute as its first Director.



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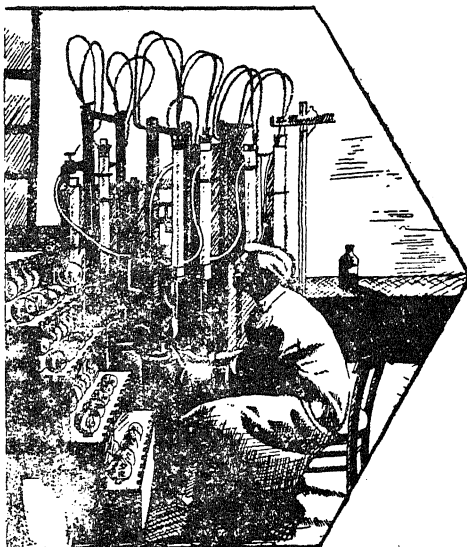
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# SOME METHODS OF REPRESENTATION OF PROTEIN CRYSTALLOGRAPHIC STRUCTURAL DATA

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THE data in the form of three-dimensional atomic coordinates that results from an X-ray analysis of a protein crystal form the basis for extracting different types of information about the tertiary structure of a protein molecule. Conventional method of model building modified suitably as in the Kendrew models give an overall picture of the intricate three-dimensional architecture. The complete specification of a molecule by the three-dimensional Cartesian atomic coordinates may be replaced in principle by a different set of parameters such as the conformational angle parameters. Thus for instance the set of  $\phi$ - $\psi$  angles<sup>1</sup> at each  $C^\alpha$ -atom specifies completely the relative positions of the backbone atoms in the protein chain. Other conformational parameters are also needed<sup>2</sup> to make the specification of a molecule complete, including the side chains. The use of the  $\phi$ - $\psi$  parameters is convenient for the description of the chain folding and is used widely, in particular, in the various approaches to prediction of protein conformations by theoretical and semi-empirical methods<sup>3</sup>. The three-dimensional models are at times felt to be unwieldy. The  $\phi$ - $\psi$  diagram although is simple and powerful, is not convenient for certain purposes, such as when one wishes to find which parts of the molecule are close to each other and so on. The so-called distance map<sup>4,5</sup> can yield this information and has been found more useful in drawing certain conclusions on the evolutionary correlations<sup>6</sup> in proteins through similarities in their patterns. Other types of analyses have also been reported for other purposes such as plotting of the distance ( $l_{i,i+3}$ ) between  $C^\alpha$  atoms separated by three residues<sup>7</sup> or the number of  $C^\alpha$ -atoms within a specified distance from a given  $C^\alpha$  atom<sup>8</sup>. Recently we have examined quite generally different types of such representation of the three-dimensional data that are possible. This is a short report of some of the main findings from our investigations. Broadly the different types of analyses may be classified into two categories of representation of the available data, namely, one- and two-parameter representations.\* The  $\phi$ - $\psi$  diagram and the

( $i-j$ ) distance map may be considered as two two-parameter representations. The chain-plot<sup>†</sup> of  $l_{i,i+3}$  is typically a one-parameter representation.

A few other possibilities we have tried are as follows: firstly, we have tried the torsion angle involving the four atoms  $C^\alpha_{i-1}$ ,  $C^\alpha_i$ ,  $C^\alpha_{i+1}$  and  $C^\alpha_{i+2}$  around the virtual bond  $C^\alpha_i - C^\alpha_{i+1}$  denoted by  $\theta_i$ , for a chain-plot. Figure 1 shows a typical chain-plot of  $\theta_i$  for myoglobin. Unlike the  $l_{i,i+3}$  plot the  $\theta$  values have a wide range, namely,  $-180^\circ$  to  $+180^\circ$  and in addition they also give information about the sense of folding of the chains as one progresses along the chain. Standard conformations such as the  $\alpha$ -helix have characteristic values of  $\theta$  which can be calculated from the standard dimensions and  $\phi$ ,  $\psi$  values of a pair of peptide units. For the  $\alpha$ -helix  $\theta$  turns out to be  $50^\circ$ . The  $\alpha$ -helical regions are seen to have fairly a constant value around  $50^\circ$  (Fig. 1).

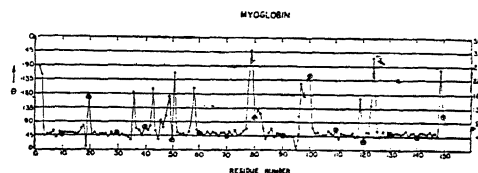


FIG. 1. Chain-plot of  $\theta_i$  for myoglobin. For convenience every tenth residue is marked by.

During these investigations we have found it convenient to evolve a few new parameters which enable us to characterise helical segments and their analysis. Some of the one- and two-parameter representations to be described below involve these new parameters.

Thus considering the  $C^\alpha$  atoms of a chain to lie on a perfect helix (i.e., with  $C^\alpha_i - C^\alpha_{i+1}$  lengths and  $\delta_i = C^\alpha_{i-1} - C^\alpha_i - C^\alpha_{i+1}$  angles all equal) a consecutive group of four atoms,  $C^\alpha_{i-1}$ ,  $C^\alpha_i$ ,  $C^\alpha_{i+1}$ ,  $C^\alpha_{i+2}$  may be considered to form the smallest segment of the helix. If  $h_i$  denotes the unit vector along the direction of the axis for the above segment, one may consider in general the directions of the axes of the different helical segments given by  $h_i$ ,  $h_{i+1}$  etc., and devise parameters for representations. For

\* In principle one should include the possible three-parameter representations also. We shall not consider these since the source itself is such a three-parameter representation and our aim is at simplified analysis.

† We use the term chain-plot to denote plotting of the value of any chosen parameter as a function of the residue number in the chain.

instance, the angle between the axes† of successive segments  $i$  and  $i+1$ , denoted by  $\eta_i$ , may be used for a chain-plot. In regions of perfect helix these values will have a constant zero value signifying perfect alignment of successive segments. A typical  $\eta_i$  plot for myoglobin is shown in Fig. 2.

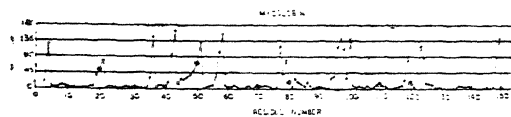


FIG. 2. Chain-plot of  $\eta_i$  for myoglobin. For convenience very tenth residue is marked by.

With the available axial directions  $h_i, h_j$  one may also plot the interaxial angles  $\eta_{ij}$  for two segments  $i$  and  $j$  and is thus a two-parameter representation. This is somewhat similar to the  $l_{i,j}$  distance map excepting that the parameter used now is an angle. Figure 3 shows the  $\eta_{ij}$  map for myoglobin.  $\alpha$ -helical regions are characterised by triangular blocks along the diagonal.

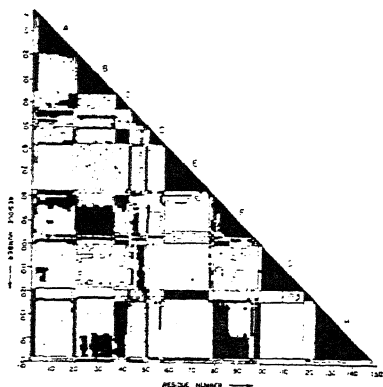


FIG. 3.  $\eta_{ij}$ -plot for myoglobin. The various helical segments A, B, etc., are marked along the diagonal. Dark, gray and blank regions correspond respectively to  $\eta$  ranges of  $0^\circ$  to  $60^\circ$ ,  $60^\circ$  to  $120^\circ$  and  $120^\circ$  to  $180^\circ$ .

The other representation may be described more appropriately as a two-dimensional representation. Here one makes use of the axial direction  $h_i$  for the various segments and plots a stereographic projection, well known to crystallographers. This projection has the advantage that the relative angles

of directions come out conveniently while the information about distances in the structure completely disappear. Thus for instance the relative angular orientation of say  $\alpha$ -helical segments can be readily discerned (Fig. 4). Ideally for a

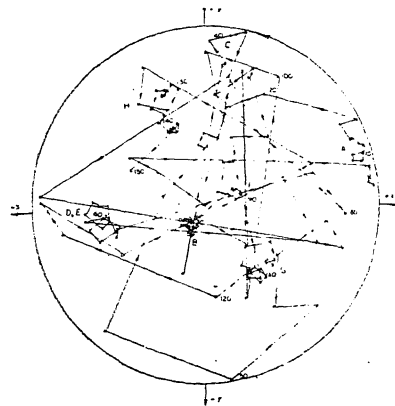


FIG. 4. Stereographic projection for  $h_i$  in myoglobin with  $+Z$  pointing upwards. Helical regions A, B, etc., are marked near respective segments.

perfect helix the projection of points corresponding to successive segments should superpose in projection but in practice one obtains only a dense distribution about a point corresponding to the mean direction. The density of packing of the points also indicates the relative tightness or perfection of the helices (e.g., compare helix E and B in Fig. 4).

Further investigations are in progress and will be reported in due course.

#### ACKNOWLEDGEMENT

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† In an actual case of a protein, a segment of four atoms  $C^{\alpha}_{i-1}, C^{\alpha}_i, C^{\alpha}_{i+1}, C^{\alpha}_{i+2}$  need not form a part of a regular helix. Still a helical axis direction  $h_i$  for such a segment can be found as a first approximation and used for our purposes.

# N<sup>4</sup>-COORDINATED ISOMER OF BIS (4-IMINO-2, 3-PENTANEDIONE-3-OXIME) PALLADIUM (II)

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## ABSTRACT

The isomer Pd(IAI)<sub>2</sub> has been isolated for the first time either by refluxing the asymmetrically coordinated palladium complex, Pd(IAI)(IAI') (where, IAI and IAI' denote N-coordinated and O-coordinated isonitrosoacetylacetonate imine respectively) or by constituents combination method at elevated temperature for a long time. Magnetic and spectral data suggest that the complex has a square planar stereochemistry around the metal-ion with both the ligands coordinated through nitrogen of the isonitroso groups.

## INTRODUCTION

**I**SONITROSO- $\beta$ -KETO imine ligands constitute an interesting class of systems, because of their ability to form isomers with 'intramolecular' as well as 'intermolecular' chelate linkage. Isonitrosoacetylacetonate imine, for example, can form three chelate linkage isomers as shown in Fig. 1. These

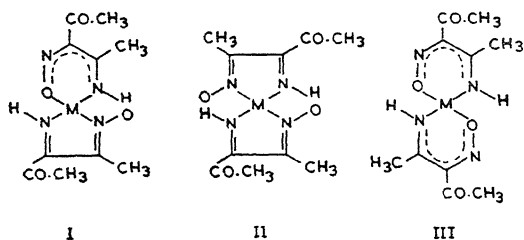


Fig. 1

isomers involve O- or N-donors of the isonitroso group and the imine nitrogen as coordination sites.

Nickel (II)<sup>1</sup>, palladium (II)<sup>2</sup> and copper (II)<sup>3</sup> are shown to form complexes of the type I with isonitrosoacetylacetonate imine. Recently, such a structure has been confirmed by x-ray diffraction studies<sup>4</sup>. There is no report so far, on the isolation of either of the isomers II or III. However, the possibility of formation of isomer II of palladium (II) in chloroform medium has been indicated spectroscopically. In this paper, we report the isolation and characterisation of the isomer of the type II for the first time with palladium (II).

## EXPERIMENTAL

Isonitrosoacetylacetonate imine was prepared by the method of Wolff *et al.*<sup>5</sup>. Palladium (II) chloride was obtained from Arora-Methey, Ltd., Calcutta. Chloroform (A.R.), a B.D.H. product, was used without further purification. Bis (isonitrosoacetylacetonate imino) palladium (II) was prepared by the reported method<sup>5</sup>. Magnetic susceptibility of the complex was determined by the Gouy method using Hg [Co(NCS)<sub>4</sub>] as the standard. I.R. spectra in Nujol mull were recorded on a Carl-Zeiss UR-10 spectrophotometer, equipped with LiF, NaCl

and KBr optics. The electronic spectra of the complexes in mull were recorded on a Unicam 700-A recording spectrophotometer.

## PREPARATION OF THE COMPLEX

The complex, Pd(IAI)<sub>2</sub>, was prepared by the following two methods.

**Method I.**—The asymmetrically coordinated red palladium complex, Pd(IAI)(IAI') (0.2 g), was taken in chloroform (400 ml) and refluxed over a water-bath for about 16 hours when the solid suspension slowly dissolved to yield a yellow solution, which on cooling gave a brown complex. It was filtered, washed with chloroform and dried in air.

**Method II.**—Palladium (II) chloride (0.2 g), isonitrosoacetylacetonate (0.2 g) and ammonia (5 ml) were taken in 500 ml chloroform and refluxed over a water-bath for about 50–55 hours. The resulting yellow solution on cooling gave a brown complex. It was filtered, washed with chloroform and dried in air.

The complex prepared by both the methods was shown to be identical as indicated by their colour, infrared spectra and chemical analysis.

## RESULTS AND DISCUSSION

The diamagnetic nature of the complex suggests square planar stereochemistry of the complex around palladium (II). The I.R. spectrum of the asymmetrically coordinated palladium (II) complex, Pd(IAI)(IAI') shows two non-coordinated C=O bands at 1684 cm<sup>-1</sup> and 1655 cm<sup>-1</sup> and two N-H bands at 3280 cm<sup>-1</sup>, 3210 cm<sup>-1</sup> (sh). The brown complex obtained by refluxing this compound in chloroform shows a single carbonyl and N-H stretching bands at 1655 cm<sup>-1</sup> and 3260 cm<sup>-1</sup> respectively in its infrared spectra. This observation suggests the intramolecular linkage isomerisation to symmetrically coordinated isomer either II or III. In deciding between these two isomers, the positions of carbonyl and N-H bands are important since these bands are shown to vary considerably depending upon the mode of coordination of the isonitroso group. The position of the carbonyl

band observed in the brown complex corresponds to the lower band at  $1650\text{ cm}^{-1}$  of  $\text{Pd}(\text{IAI})(\text{IAI}')$ . This band has been assigned to the N-coordinated isonitroso- $\beta$ -keto imine. It is important to note here that the  $\text{C}=\text{O}$  frequency of the O-coordinated isonitroso- $\beta$ -keto imine ligand occurs at a higher frequency compared to the N-coordinated isonitroso- $\beta$ -keto imine ligand. The band at  $3260\text{ cm}^{-1}$  assigned to N-H stretching mode of  $\text{Pd}(\text{IAI})_2$  agrees well with the higher frequency band observed in the I.R. spectra of the asymmetrically coordinated palladium(II), nickel(II) and copper(II) complexes of the same ligand. This band is also assigned to the N-coordinated isonitroso- $\beta$ -keto imine ligand. These evidences suggest that both the ligands are coordinated to palladium(II) through nitrogen donors of the isonitroso groups in the brown complex.

The proposed structure for the complex is further supported by comparing its electronic spectrum with that of  $\text{Pd}(\text{IAI})(\text{IAI}')$  in the solid state. The assignment of the electronic spectral bands of  $\text{Pd}(\text{IAI})_2$  are given in Table I.

Unlike  $\text{Pd}(\text{IAI})_2$ , which shows four bands in the visible region,  $\text{Pd}(\text{IAI})(\text{IAI}')$  shows a broad shoulder around  $21200\text{ cm}^{-1}$ , which may possibly be the expected  $d-d$  transition. The differences in

the electronic spectra of the two complexes also support the proposed structure for the former complex.

TABLE I  
Electronic spectral bands of  $\text{Pd}(\text{IAI})_2$  in mull

| $\nu$ in $\text{cm}^{-1}$ | Assignment                               |
|---------------------------|--|
| 22700                     | Possibly a charge transfer band          |
| 20600                     | $1A_{1g} \rightarrow 1E_{1g}(\text{sh})$ |
| 18500                     | $1A_{1g} \rightarrow 1B_{1g}$            |
| 17000                     | $1A_{1g} \rightarrow 1A_{2g}$            |

The isolation of the present complex shows that the kind of chelate linkage isomer formed by the isonitroso- $\beta$ -keto imine ligands depends upon the physical conditions like temperature, solvent, etc.

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### 3, 5-DICHLORO-2-HYDROXYACETOPHENONE OXIME AS A CHELATING AGENT: STUDIES ON ITS PALLADIUM(II) CHELATE

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#### ABSTRACT

3, 5-Dichloro-2-hydroxyacetophenone oxime (DCHAO) has been found to be a good reagent for gravimetric estimation of palladium and for its separation from other ions. The composition and structure of the chelate are studied by microanalysis, pH metric titration, I.R. and Electronic spectra.

**O**-HYDROXY keto-oximes have been successfully used as chelating reagents. The gravimetric and spectrophotometric studies of the metal chelates in solution have been reported by several workers<sup>1-4</sup>. Gupta and Lal<sup>5-7</sup> have reported the physico-chemical studies on the chelates of Cu(II), Ni(II) and Co(II) with 3, 5-dichloro-2-hydroxyacetophenone oxime (DCHAO). In this communication, we report DCHAO as a gravimetric reagent for Pd(II). The composition and structure of Pd(II) chelate has been determined on the basis of micro-analysis, pH metric titration, I.R. and Electronic spectra.

#### EXPERIMENTAL

DCHAO was prepared as reported earlier<sup>8</sup>. An ethanolic solution (0.5%) of the ligand was

employed for the gravimetric studies. A solution of palladium chloride was prepared from B.D.H. (A.R.) sample in 0.05 M HCl and standardised gravimetrically. Solutions of other ions were prepared from reagent grade samples. NaOH solution (0.05 M) was used for the pH titration using systronic pH meter (type 322).

#### Determination of Palladium (II)

Metal ion solution (~20 mg) is diluted to 100-125 ml and the pH adjusted (1.0 to 5.0) with hydrochloric acid and ammonium hydroxide buffer, heated to 60-70° and treated with (0.5%) ethanolic solution of DCHAO dropwise with constant stirring (about double the theoretical amount). The precipitate (yellow) was digested on water-bath

for about half an hour. It was filtered while hot through sintered glass crucible (G-4), washed with hot water and finally with 60% ethanol, dried at 100–120° C to a constant weight. It has been found that palladium can be quantitatively determined in the pH range 1.5–4.0. The gravimetric factor (metal/metal complex) is 0.19544.

#### Study of Interference

The procedure was the same as in the absence of foreign ions. The interference of Cu (II) and Fe (III) has been removed by using EDTA and tartaric acid (masking agent) respectively. Excess (10–15 times) of cations like  $\text{UO}_2$  (II), Ni (II), Co (II), Zn (II), Cd (II), Mo (VI), Hg (II), Mn (II), Sb (III), Bi (III), As (III) and sufficiently large excess of anions like  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{C}_2\text{O}_4^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{PO}_4^{3-}$  citrate and tartrate do not interfere at pH 2.0.

From the solution containing both palladium and cobalt/nickel, palladium was first precipitated as above at pH 2.5 and from filtrate, cobalt/nickel was precipitated at pH 7.5 and 6.5 respectively as reported earlier.<sup>6</sup>

#### Accuracy of the Estimation

Palladium (6–50 mg) could be estimated with an accuracy of  $\pm 0.4\%$ . At lower concentrations of the metal (4–6 mg) the error was about 4% higher.

#### Analysis

Elemental analysis for  $\text{Pd}(\text{C}_8\text{H}_6\text{NO}_2\text{Cl}_2)_2$ : calculated N 5.14, Cl 26.08 and Pd 19.54%, found N 5.17, Cl 26.04 and Pd 19.58%.

#### pH Metric Titration

The titrations were carried out with the solution containing metal and the ligand in the ratio 1 : 0, 1 : 1, 1 : 2 and 1 : 3 with 0.05 M NaOH.

Figure 1 represents the change of pH with the addition of alkali to various systems. Since palladium solution was prepared in 0.05 M HCl, necessary correction was made while evaluating the composition. Curve A (1 : 0, metal : ligand) shows two inflexions at 5 and 7 moles of alkali due to complete neutralization of acid and precipitation of metal as hydroxide respectively.

Curve B (1 : 1, metal : ligand) shows a lowering of pH due to the liberation of proton from the hydroxyl group on complexation with palladium. It is assumed that (1 : 2, metal : ligand) complex is formed, hence one proton released and half of the metal ion remains in free state. Both the released proton and free metal require two moles of alkali and five moles of alkali are needed for the acid, therefore one inflexion is observed at about 7 moles of alkali. Curves C and D are also very similar

to Curve B and confirm the 1 : 2 complex formation in this case.

Metal ligand ratio is also determined by Fenger *et al.*<sup>8</sup> method and found to be 1 : 2.

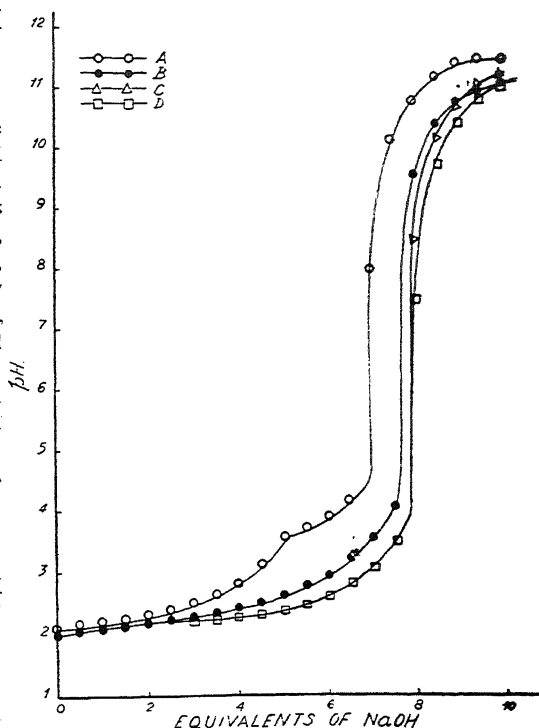


Fig. 1. pH metric titration of palladium chloride (5 ml,  $1 \times 10^{-2}$  M in 0.05 M HCl) with (0.05 M) NaOH. Curves A, B, C and D obtained in the presence of 0, 5, 10 and 15 ml of ligand (0.01 M) respectively, total volume 25 ml.

#### I.R. Spectra

I.R. spectra of the ligand and chelate were recorded from 4000–400  $\text{cm}^{-1}$ . In the ligand, two bands at 3333 and 3060  $\text{cm}^{-1}$  are assigned to intramolecularly bonded hydroxyl and aromatic C–H stretching frequencies respectively<sup>9</sup>, which shifts to 2850  $\text{cm}^{-1}$  in the chelate. The bands observed at 1580  $\text{cm}^{-1}$  and 1560  $\text{cm}^{-1}$  are due to  $\text{C}=\text{N}$  stretching and ortho substituted benzene ring vibration in the ligand. These two bands are coupled with each other and it seems reasonable to assume that the peak at 1560  $\text{cm}^{-1}$  is due to  $\text{C}=\text{N}$  stretching vibration, since these bands are lowered to 1532  $\text{cm}^{-1}$  in the chelate. The frequency arising at 1645  $\text{cm}^{-1}$  is ascribed to O–H deformation mode which is in good agreement with the results obtained by Ramaswamy *et al.*<sup>9</sup>. Very strong band at 1260  $\text{cm}^{-1}$  can be assigned to N–O stretching, and this shifts to 1230  $\text{cm}^{-1}$  in the chelate. Blinc and Hadzi<sup>10</sup>

explained the shift of N—O frequency to higher region on the basis of the contribution of polar structure of the ligand. The metal-nitrogen and metal-oxygen stretching vibrations have been recorded at 520 and 580  $\text{cm}^{-1}$  respectively.

Thus it is clear that on chelation hydrogen of hydroxyl group is replaced and nitrogen of oximino group donates a lone pair of electron to the metal ion.

### Electronic Spectra

The ground state for the low spin complexes of  $d^7$  configuration is

$$[a_{1g}(\pi^*)]^2 [e_g(xy, yz)]^4 [b_{2g}(xy)]^2 \equiv {}^1A_{1g}$$

and are diamagnetic. The present DCHAO complex is also diamagnetic in nature. Three spin allowed  $d-d$  transitions are anticipated corresponding to transitions from the three lower lying  $d$ -levels to the empty  $d_{x^2-y^2}$  orbital; two electron transitions would be very weak and neglected. Energies corresponding to these transitions are determined from the following equations<sup>11</sup>:

$$d_{xy}(b_{2g}) \longrightarrow d_{x^2-y^2}(b_{1g}) E({}^1A_{1g} \longrightarrow {}^1A_{2g}) \\ = \Delta_1 - C$$

$$d_{xy, yz}(e_g) \longrightarrow d_{x^2-y^2}(b_{1g}) E({}^1A_{1g} \longrightarrow {}^1E_g) \\ = \Delta_1 + \Delta_2 - (3B + C)$$

$$d_{z^2} \longrightarrow d_{x^2-y^2}(b_{1g}) E({}^1A_{1g} \longrightarrow {}^1B_{1g}) \\ = \Delta_1 + \Delta_2 + \Delta_3 - (4B + C)$$

Only two bands at 32010  $\text{cm}^{-1}$  and 44500  $\text{cm}^{-1}$  have been observed in the electronic spectra of  $\text{Pd}(\text{C}_5\text{H}_6\text{NO}_2\text{Cl}_2)_2$ . Following the assignment of Mason and Gray<sup>12</sup>, on the electronic spectra of the square planer  $[\text{Pd}(\text{NH}_3)_4]^{2-}$  complex, we may

presume that the former one is a combination of all the three spin allowed transitions. This means that in the case of  $\text{Pd}(\text{II})$ , complex, the values of ligand field parameters  $\Delta_1$ ,  $\Delta_2$  and  $\Delta_3$  derived from the  $d-d$  spectra of  $\text{Pd}(\text{II})$  complex were found 35510, 1500 and 500 (in  $\text{cm}^{-1}$ ) respectively taking  $B = 500 \text{ cm}^{-1}$ ,  $C = 3500 \text{ cm}^{-1}$ . The other band at 44500  $\text{cm}^{-1}$  is due to the  ${}^1A_{1g} \longrightarrow {}^1E_g$  transition<sup>13</sup>.

### ACKNOWLEDGEMENT

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## A SIMPLE AND ELEGANT TECHNIQUE TO ASCERTAIN FOOD ACCEPTABILITY AND MIGRATORY HABITS OF EARTHWORMS

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LITERATURE survey reveals that earthworms can use a wide variety of organic materials for their nutrition and propagation. The author and his colleagues had maintained in the laboratory colonies of earthworms for many years on a mixture of hay, weeds, kitchen waste and compost (which included animal dung)<sup>1</sup>. Evans and Guild<sup>2</sup> reported that earthworms produced more cocoons when fed on decaying animal products than those fed on plant materials. That earthworms can thrive well on animal dung has been known for a long time<sup>3,4</sup>. In fact, cowdung has been observed

to be superior to kitchen waste, straw, green manure, or even a mixture of all the four for the propagation of earthworms as well as from the point of view of increase in azotobacterial population, the latter being of presumable importance for the fixation of nitrogen in soil<sup>4</sup>.

Mention may be made in this context that Darwin<sup>5</sup> had reported on the behaviour of earthworms in relation to their selection of leaves for food as well as the manner in which the selected leaves get pulled to their burrows. It is known that earthworm can distinguish between different kinds of

plant litter and that they even show preference for particular shapes<sup>5</sup>, leaf species<sup>6</sup>, and for those species which are rich in protein<sup>7</sup>. From the experimental evidence it would appear that earthworms do react fairly strongly to chemical stimuli as such. In other words, their food preference is dependent on chemoreception.

The present investigation was undertaken with a view to ascertaining which of the plant leaves available in abundance in this region can serve as suitable feed material for the earthworm and to make sure if the animal would prefer foliage to cowdung which had previously been established as an adequate diet.

The technique evolved, after several experimentations, proved to be simple and elegant and is presented here.

#### EXPERIMENTAL AND RESULTS

The acceptability or otherwise of foliage was tested in trays measuring 39 cm long, 28 cm wide and 6 cm deep. These trays have been found satisfactory for maintaining colonies of earthworms in the laboratory for months together and/or studying their population dynamics or for other investigations. Also, clean sand has been found to be a suitable medium to mix with dietary materials for carrying out nutritional experiments on this animal.

For the present experiment, each tray was marked into three portions, 13×28 cm each. At one end portion was maintained a colony of earthworms (*Pheretima* species) raised in the local soil as the habitat and cowdung as the feed material. The feed was given only once at the commencement of the experiment. The other end portion of the tray was filled with clean sand collected from the sea-shore and in it were layered the leaves to be tested for their suitability or otherwise as the diet. With tap water, the soil at one end and sand at the other were levelled and smoothened at surfaces with glass slide and left overnight.

The following day the activity of the earthworms overnight could be discerned from their burrows and castings thrown on the surface. The number of worms in the original colony and the number which had migrated into the sand bed was counted, whenever any migration had occurred. Leaves from the sand bed were then individually and carefully examined to note down the part or parts of the leaves nibbled at by the worms. All the worms from the sand were again transferred into the original colony. At the end of the day, the soil bed at one end and the sand bed at the other layered with leaves were again levelled with water and left overnight for earthworms to migrate. The following day the numbers migrated were again

counted and the experiment thus continued with periodic migratory records for nearly seven weeks. In this way, not only their migratory habits towards preferable or otherwise foods, or adherence to the available food—cowdung—in their resident colony were recorded.

It was interesting to note that in certain instances the worms did not migrate at all, whereas in others, they exhibited their preference for the foliar diet most eloquently. In the following table, by no means comprehensive or complete, are presented some of the typical results recorded for only eight of the several plant species foliage put to the test.

TABLE I

Acceptability or otherwise of foliage by earthworms

| Plant leaf used              | No. of worms in the parent colony | No. of worms migrated after days |    |    |    |    |    |    |
|------------------------------|-----------------------------------|----------------------------------|----|----|----|----|----|----|
|                              |                                   | 6                                | 9  | 11 | 16 | 29 | 34 | 36 |
| <i>Eugenia corymbosa</i>     | 40                                | 12                               | 10 | 13 | 9  | 10 | .. | .. |
| <i>Colacasia antiquorum</i>  | 20                                | ..                               | 11 | 15 | .. | .. | .. | .. |
| <i>Jatropha curcas</i>       | 149                               | 9                                | 50 | 52 | 60 | 66 | .. | 60 |
| <i>Eugenia jambolana</i>     | 22                                | ..                               | .. | .. | .. | .. | .. | 3  |
| <i>Cassia tora</i>           | 32                                | 3                                | 3  | 4  | 1  | 15 | 9  | 12 |
| <i>Polyalthia longifolia</i> | 22                                | ..                               | .. | .. | .. | .. | .. | .. |
| <i>Achras sapota</i>         | 111                               | 20                               | 10 | 20 | 27 | .. | .. | .. |
| <i>Psidium guajava</i>       | 20                                | ..                               | .. | .. | .. | 2  | 3  | .. |

The observation that for as many as five days, the worms did not migrate from the parent colony towards the foliar diet is suggestive of the adequacy of available food in the parent colony itself. Cowdung diet was, it may be recalled, supplied only once at the commencement of the experiment. Thus, after five days partly due to the diminishing food supply in the colony and perhaps partly due to chemoreception, their migration (towards foliage supplied) became observable. Preference for foliar diet is, it may be pointed out, dependent on chemoreception and the presence of polyphenols and alkaloids components therein which markedly influence the preference or otherwise<sup>8,9</sup> by the worms. This was clearly evidenced in this as well as other experiments carried out. For example, mango leaf (*Mangifera indica*) is not acceptable at all to the worms in either fresh or partly decomposed state. In fact, the worms died when attempts were made (unpublished data) to grow them in a diet solely comprised of mango leaf, whereas the worms thrived very well on other foliar diets, e.g., in partly decomposed guava leaves. On the other



hand, as may be seen from the above results, *P. guajava* leaves do not attract them for as long as a month and, what is more interesting, even after a few had migrated, the leaves were not nibbled at, by the animals.

It is also clear from the above results that *Polyalthia longifolia* leaves were not acceptable as no worm ever migrated towards them for as long as 36 days. *Cassia tora* leaves, on the other hand, were observed to be a preferred food whether in fresh state or after partial decomposition. *Jatropha curcas* foliage also was an acceptable diet. *Eugenia jambolana* did not prove to be attractive enough, as the worms failed to migrate to them for several days. Even when they did, only a small number migrated. However, they nibbled at the food, perhaps reluctantly. Their attraction towards *Colacasia antiquorum* was for a short period.

The above experiment thus provided an easy means of detecting acceptability or otherwise of foliage by the experimental animal—earthworms.

#### ACKNOWLEDGEMENTS

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## LETTERS TO THE EDITOR

THE NEAR ULTRAVIOLET ABSORPTION  
SPECTRA OF 2, 6- AND 3, 5-DICHLORO-  
BENZONITRILES

THE vibrational analysis of the infrared and ultraviolet absorption spectra of benzonitrile has been carried out by Hert and Howe<sup>1</sup> and Bass<sup>2</sup>. The effect of monohalogen substitution on the spectra of benzonitrile has been extensively studied by other

obtained in the two molecules have been given in Table I. These fundamentals have also been correlated with the infrared fundamentals<sup>3</sup>. The assignments to these fundamentals have been made on the basis of correlation with other substituted benzonitriles. All the observed bands have been explained as the combination of fundamentals and  $\nu$ - $\nu$  transitions and their overtones.

TABLE I

| 2, 6-DCBN             |         |         | 3, 5-DCBN             |         |         | Assignment   |
|-----------------------|---------|---------|-----------------------|---------|---------|--|
| G.S.                  |         | E.S.    | G.S.                  |         | E.S.    |  |
| I.R. $\text{cm}^{-1}$ | U.V. nm | U.V. nm | I.R. $\text{cm}^{-1}$ | U.V. nm | U.V. nm |  |
| 275                   | 255     | ..      | 258                   | ..      | ..      | C-Cl, O.P. bending                                   |
| 404                   | ..      | 290     | 458                   | 446     | 420     | $a_1$ component of $e_{2g}$ (608 $\text{cm}^{-1}$ )  |
| 716                   | ..      | 528     | 662 }<br>717 }        | ..      | ..      | C-Cl, stretching                                     |
| 983                   | ..      | 923     | 994                   | ..      | 967     | C-C-C, trigonal bending                              |
| 1433                  | ..      | 1183    | 1412                  | ..      | 1220    | $a_1$ component of $e_{1u}$ (1485 $\text{cm}^{-1}$ ) |

investigators<sup>3-8</sup>. In the present communication, the near ultraviolet absorption spectra of 2, 6- and 3, 5-dichlorobenzonitriles (abbreviated as 2, 6-DCBN and 3, 5-DCBN) are reported.

The ultraviolet absorption spectra of both the molecules were photographed in the vapour phase on a Hilger medium quartz spectrograph. The hydrogen arc lamp was used as the source of continuous radiation. Well-defined absorption bands were obtained with a path length of 225 cm at the vapour temperature 20° to 100° C.

Both the molecules have  $C_{2v}$  symmetry. The ultraviolet absorption spectra of both molecules were found to be due to the  $A_1 - B_1$  transition which corresponds to the  $A_{1g} - B_{2u}$  transition of benzene. The strong band at 34015  $\text{cm}^{-1}$  and 34262  $\text{cm}^{-1}$  in 2, 6- and 3, 5-DCBN respectively have been taken as 0, 0 band. About 37 bands have been observed for 2, 6-DCBN and 55 bands for 3, 5-DCBN. The bands are degraded towards red but are sharp in general. The ground and excited state fundamentals

TABLE II

| Molecules                                 | Position of 0, 0 bands in $\text{cm}^{-1}$             |      |
|---|--|------|
|   | Shift with respect to benzonitrile in $\text{cm}^{-1}$ |      |
| Benzonitrile <sup>1,2</sup>               | 36516  | ..   |
| <i>o</i> -chlorobenzonitrile <sup>6</sup> | 35156  | 1360 |
| <i>m</i> -chlorobenzonitrile              | 35239  | 1277 |
| <i>p</i> -chlorobenzonitrile <sup>4</sup> | 36327  | 189  |
| 2, 6-DCBN                                 | 34015  | 2401 |
| 3, 5-DCBN                                 | 34262  | 2254 |

Present work

Table II shows the position of 0, 0 band in *o*-, *m*- and *p*-chlorobenzonitriles and in the present mole-

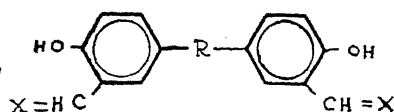
cules. The red shift in *o*-, *m*- and *p*-chlorobenzonitriles is in the order of  $o > m > p$ -CBN. In the present molecules, red shift is in the order of  $2,6 > 3,5$ -DCBN. From these observations, it can be concluded that as the distance of substituent atom from nitrile (CN) group increases, 0,0 band shifts towards the shorter wavelengths.

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### NICKEL CHELATES OF 5-SUBSTITUTED SALICYLALDEHYDE AND 5-SUBSTITUTED SALICYLALDOXIME LIGANDS

CHELATE polymers of bis-bidentate salicylaldehyde derivatives such as salicylaldehyde derivative (I-a) and salicylaldimine derivative (I-b) have been well investigated<sup>1-4</sup>. We present here our studies on the chelates of nickel with salicylaldehyde derivative (I-c) and salicylaldoxime derivative (I-d).



(I)

|        |   |
|--------|---|
| X =    | R =   |
| a. O   | CH <sub>2</sub> ; SO <sub>2</sub>   |
| b. NH  | CH <sub>2</sub> ; SO <sub>2</sub>   |
| c. O   | N <sub>2</sub> C <sub>6</sub> H <sub>4</sub> C <sub>6</sub> H <sub>4</sub> N <sub>2</sub> |
| d. NOH | N <sub>2</sub> C <sub>6</sub> H <sub>4</sub> C <sub>6</sub> H <sub>4</sub> N <sub>2</sub> |

**Experimental Procedure.**—4-4' bis (m-formyl-*p*-hydroxy phenyl azo) biphenyl (H<sub>2</sub> BASA) and its dioxime (H<sub>2</sub> BASAO) were prepared by the method of Sen and Ghosh<sup>5</sup>.

**Nickel chelates of 4-4' bis (m-formyl-*p*-hydroxy-phenyl azo) biphenyl and its dioxime (HBASA)<sub>2</sub> Ni and (BASAO)<sub>2</sub> Ni.**—When the solution of nickel acetate in pyridine was mixed with the solution of the ligand (H<sub>2</sub> BASA or H<sub>2</sub> BASAO) in pyridine (salt : ligand : : 1 : 1), the mixture became turbid,

slowly forming slimy precipitates when left overnight. The precipitates were filtered, washed with a little pyridine and alcohol and dried. They were insoluble in water and in all common organic solvents except dimethyl formamide and pyridine in which they were slightly soluble.

Magnetic susceptibility (X<sub>g</sub>) of these chelates was determined on Gouy's magnetic balance at room temperature. Thermal analysis (T.G.A.) of the chelates was carried out in nitrogen atmosphere using Lenseis Model. Visible absorption spectra of the nickel chelates in dimethyl formamide were measured on a Spekol spectrophotometer.

### Discussion

(HBASA)<sub>2</sub> Ni.—The chelate is orange-red in colour and does not melt upto 300° C. The stoichiometry of the chelate indicates the ratio of metal to nitrogen as 1 : 9 [Analysis : Found : % Ni : 5.42 ; % N = 11.52 (C<sub>57</sub>H<sub>47</sub>N<sub>9</sub>O<sub>12</sub>) Ni required % Ni : 5.34 ; % N : 11.47]. Further it gives the smell of pyridine on heating. Hence it is formulated as monomeric nickel chelate Ni (HBASA)<sub>2</sub> py aq. Nickel is not precipitated from its dilute solution in dimethyl formamide by hydrogen sulphide. The solution gives the visible absorption spectrum which is characteristic of an azo compound.

The stepwise weight loss observed in T.G.A. studies indicates that the chelate (i) loses pyridine and part of water over a temperature range of 70–150° and (ii) loses the remaining amount of water sharply at 475–80° C. The total weight loss observed is about 14%. The residue left in this study is anhydrous nickel chelate but upto 475° C, the two water molecules are strongly held by the molecule, forming octahedral chelate molecule.

Its magnetic susceptibility is found to be  $2.8 \times 10^{-6}$  C.G.S. unit at 29° C. Hence magnetic moment per nickel atom is calculated as 2.86 B.M. It confirms the octahedral nature of the chelate.

Although we could not prepare the anticipated thermally stable polychelate, the monomeric chelate obtained is quite stable thermally.

(BASAO)<sub>2</sub> Ni.—It is orange red in colour and does not melt upto 300° C. The stoichiometry of the chelate indicates the ratio of metal to ligand as 1 : 1 [Analysis : Found : % Ni = 10.31 ; % N = 15.13. (C<sub>26</sub>H<sub>20</sub>N<sub>6</sub>O<sub>5</sub>) Ni required % Ni = 10.53 ; N = 15.15]. It gives no smell of pyridine on heating. Hence it is formulated as linear chain [Ni(BASAO)aq]<sub>n</sub>.

Nickel is not precipitated from its dilute solution in dimethyl formamide by hydrogen sulphide. The solution gives the visible absorption spectrum which is characteristic of an azo compound.

The stepwise weight loss observed in T.G.A. studies indicates that there is (i) loss of water associated with the decomposition of the chelated ligand at low temperatures (90–170°C), and (ii) drastic decomposition above 300°C. The low temperature decomposition appears to involve the loss of nitrogen. We find that although the polychelate could be obtained, it has low thermal stability.

The magnetic susceptibility of the chelate is found to be  $5.12 \times 10^{-6}$  C.G.S. unit at 29°C. Hence magnetic moment of the chelate per nickel atom is calculated at 2.75 B.M. It indicates octahedral nature of the chelate. Taking into consideration the formula and low value of the magnetic moment, we suggest the interlinking of chains, leading to some spin-spin interaction.

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## NUCLEAR MAGNETIC RESONANCE STUDIES OF HYDROGEN BONDING

THE present paper deals with the results obtained by NMR spectroscopic studies of the equilibrium constants for the systems pyrazine, 2,2'-bipyridyl and 1,10-phenanthroline as proton acceptors with methanol, ethanol and piperidine as donors. Incidentally, the equilibrium constants for the chloroform-pyrazine, chloroform-acetone, hydrogen bonded systems have been reported<sup>1,2</sup>.

### Experimental

B.D.H. spectrograde carbon tetrachloride, cyclohexane and chloroform were used after further purification by standard methods<sup>3</sup>. E. Merck grade 2,2'-bipyridyl was used after recrystallizing from benzene and cyclohexane. E. Merck grade

1,10-phenanthroline monohydrate is dehydrated at 105°C and then recrystallized from benzene and then from carbon tetrachloride. It had an m.p. 117°C<sup>4</sup>. Fluka grade pyrazine was used without further purification.

All the spectra were recorded employing Varian Associates A 60-D NMR spectrometer (Analytical Instrument) operating in the field sweep mode equipped with temperature control. Chemical shifts of the Lewis acids were measured relative to TMS or cyclohexane. The precision of the chemical shifts is  $\pm 0.5$  Hz. The probe temperature is  $37 \pm 0.5^\circ\text{C}$ . Each NMR tube was fitted with a cap to prevent evaporation of the solvent.

### Procedure

The hydrogen bonding chemical shifts for the alcohols and piperidine were determined along with K by measuring the chemical shifts of the -OH proton of alcohols and NH proton of piperidine under very dilute conditions of these Lewis acids and varying amounts of donors.

In order to evaluate the equilibrium constant (K) and the H-bonding chemical shift ( $\Delta W^2$ ), the following relations were used<sup>5-7</sup>:

$$\begin{aligned} 1/\Delta W_{\text{observed}} &= 1/K \cdot \Delta W^2 [B]_0 - 1/\Delta W^2 \quad (1) \\ \Delta W_{\text{observed}} [B]_0 &= -\Delta W_{\text{observed}} \cdot K - \Delta W^2 \cdot K \quad (2) \end{aligned}$$

where K is the equilibrium constant,  $[B]_0$  the initial concentration of the base,  $\Delta W_{\text{observed}}$  is the difference between the observed chemical shift and that of free acid and  $\Delta W^2$  the difference between free and complexed acid. All the computations were carried out in a single operation on an IBM 1130 computer using the general least squares method.

### Results and Discussion

The hydrogen bonding chemical shifts  $\Delta W^2$  for methanol, ethanol, piperidine with a series of Lewis bases and the corresponding equilibrium constants are given in Table I along with acid concentration and base concentration range. Measurements were made on five concentrations in each case.

The concentrations of acids, methanol, ethanol, piperidine and chloroform are such that the self-association does not compete with the given Lewis bases<sup>10-13</sup>.

From the results of Table I it can be seen that variations in the estimates of  $\Delta W^2$  using equation (1) obviously affect the value of K. The modified equation (2) directly gives K and hence the values of K so obtained may reasonably be accepted to be more accurate than those obtained with equation (1)<sup>9</sup>.

TABLE I

| Sl. No. | Lewis acid | Lewis base          | Acid concentration | Base concentration range | From W° | equation (1) K.1/mol. | From W° | equation (2) K.1/mol. |
|---------|------------|---------------------|--------------------|--------------------------|---------|-----------------------|---------|-----------------------|
| 1.      | Methanol   | Pyrazine            | 0.19708            | 0.1428-0.6422            | 136.2   | 1.22                  | 149.1   | 1.08                  |
| 2.      | Ethanol    | Pyrazine            | 0.27412            | 0.1428-0.6422            | 62.6    | 0.44                  | 42.3    | 0.59                  |
| 3.      | Piperidine | Pyrazine            | 0.16171            | 0.1428-0.6422            | 152.5   | 0.092                 | 32.5    | 0.376                 |
| 4.      | Methanol   | 2,2-bipyridyl       | 0.19708            | 0.20146-0.92672          | 214.2   | 0.98                  | 212.9   | 0.98                  |
| 5.      | Ethanol    | 2,2-bipyridyl       | 0.27412            | 0.20146-0.92672          | 108.5   | 2.04                  | 120.7   | 1.67                  |
| 6.      | Piperidine | 2,2-bipyridyl       | 0.16171            | 0.20146-0.92672          | 17.6    | 1.62                  | 21.8    | 1.14                  |
| 7.      | Methanol   | 1:10 phenanthroline | 0.19708            | 0.01663-0.07982          | 243.0   | 6.88                  | 245.7   | 6.80                  |
| 8.      | Ethanol    | 1:10 phenanthroline | 0.27412            | 0.01663-0.07982          | 276.9   | 5.00                  | 227.0   | 6.40                  |
| 9.      | Piperidine | 1:10 phenanthroline | 0.16171            | 0.01663-0.07982          | 15.07   | 8.00                  | 33.2    | 4.17                  |
| 10.     | Chloroform | Pyrazine            | 0.04998            | 0.1428-0.6422            | 138.0   | 0.105                 | 97.7    | 0.151                 |
| 11.     | Chloroform | Acetone             | 0.1247             | 0.6808-6.808             | 64.6    | 0.43                  | 64.1    | 0.43                  |

The values of K increase from pyrazine to 1:10 phenanthroline. This may be expected from their structures. Pyrazine obviously gives a weaker intramolecular hydrogen bonded complex. The two nitrogens of 1,10-phenanthroline are cis-planar which adds to the feasibility of the intramolecular exchange of hydrogen bond. Work is in progress to test this suggestion.

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**EVIDENCE FOR EXISTENCE OF +2  
OXIDATION STATE OF IRON IN  $\text{KF}_6\text{Cr}(\text{CN})_6$   
BY MÖSSBAUER SPECTROSCOPY**

THE Mössbauer effect has been extensively used to study a wide variety of ionic ferrous compounds<sup>1</sup>. The physical quantities isomer shift ( $\delta$ ) and quadrupole splitting ( $\Delta E_Q$  or  $\Delta$ ) give a direct picture of the environment around the nucleus. The displacement of resonance line from zero velocity gives the isomer shift and as the position of the resonance line is governed by the s-electron density of the iron, isomer shift value is representative of the oxidation state of the sample under investigation. In the present communication studies of the oxidation state of iron in  $\text{KF}_6\text{Cr}(\text{CN})_6$  with mössbauer spectroscopy has been reported.

**Experimental**

The mössbauer spectrum was recorded at IIT, Kanpur, employing an ND-512 multichannel analyzer. The source used was 2.0 m Ci  $^{57}\text{Co}$  in copper matrix. The IR spectrum of the compound was recorded with a Beckman IR 20 spectrophotometer (range 4000–350  $\text{cm}^{-1}$ ).

The sample was prepared by mixing aqueous solutions of potassium hexacyanochromate (III) and ferrous ammonium sulphate (B.D.H., A.R.) in their molar proportions. The orange coloured precipitate was filtered and washed with distilled water, alcohol and finally with ether. The sample was dried in vacuum over anhydrous calcium chloride. The dried sample was then ground to a fine powder and a 50  $\text{mg}/\text{cm}^2$  thick layer was sandwiched between the folds of an aluminium foil and mounted on the sample holder of the instrument.

**Analysis of the Data.**—The instrument was calibrated using enriched iron (99.99% pure). The count rate was plotted against channel number to obtain the mössbauer plot (Fig. 1).

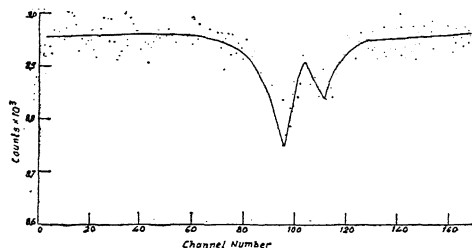


FIG.1. Mössbauer spectrum of  $\text{KFeCr}(\text{CN})_6$ .

The velocity at a particular channel was determined using a previously worked out computer programme.

**Results and Discussion**

The infra red spectra of the compound showed two  $\text{C} \equiv \text{N}$  stretching bands at 2160  $\text{cm}^{-1}$  and

2085  $\text{cm}^{-1}$  as reported by Shriver *et al.*<sup>2</sup>, and  $\text{Cr} - \text{C}$  stretching band at 600  $\text{cm}^{-1}$ .

The mössbauer spectrum of  $\text{KF}_6\text{Cr}(\text{CN})_6$  shows dips at channel numbers 96 and 112. The velocity corresponding to these channel numbers comes out to be 0.0 mm/sec. and 1.71 mm/sec.

Thus

isomer shift ( $\delta$ ): 0.85 mm/sec.

and

quadrupole splitting ( $\Delta$ ): 1.71 mm/sec.

Identification of iron as high spin ferrous or ferric can be easily carried by mössbauer spectroscopy<sup>3,4</sup> as each state of iron gives a distinctive pattern. Thus two lines appearing in the mössbauer spectrum of  $\text{KF}_6\text{Cr}(\text{CN})_6$  with a quadrupole splitting value of 1.71 mm/sec. show that iron in this compound is in ionic and +2 oxidation state.

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**REACTION OF DIETHYL- $\gamma$ -OXO-PIMELATE  
WITH ALDEHYDES. A TYPE OF STOBBE  
CONDENSATION**

THE reaction of diethyl- $\gamma$ -oxo-pimelate and anisaldehyde in the presence of potassium tert-butoxide was observed to yield an acid in 90% yield. The equivalent weight and the analytical values suggested that it was an ester-acid<sup>1</sup> and this was supported by the spectral data [ $\lambda_{\text{uv}}^{\text{EtOH}}$ : 226 nm ( $\log \epsilon$  4.16), 304–308 nm ( $\log \epsilon$  3.40); ir: 1685  $\text{cm}^{-1}$  (an extended aryl conjugated  $\alpha$ ,  $\beta$ -unsaturated carbonyl), 1718  $\text{cm}^{-1}$  (saturated carboxyl), 1735  $\text{cm}^{-1}$  (saturated ester)].

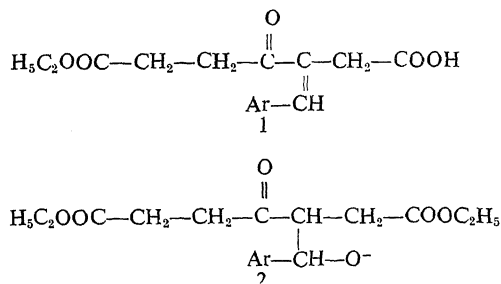
The formation of  $\beta$ -arylidene- $\gamma$ -oxo- $\omega$ -carbethoxy hexanoic acid takes place in preference to the  $\beta$ -ketoester which could be obtained by an internal self condensation corresponding to Dieckmann Cyclisation, or an acyclic diester by a Claisen reaction. The formation of the ester-acid could be explained by assuming that a carbanion, formed from the more acidic keto-methylene, condenses with the aldehyde to yield the oxyanion<sup>2</sup>. This could cyclise to a  $\gamma$ -lactone as in the Stobbe reaction<sup>1</sup>, and the lactone then cleaves to the ester-acid<sup>1</sup>.

TABLE I  
Reaction of Diethyl- $\gamma$ -oxo-pimelate with Aryl aldehydes

| Sl. No. | Ar   | mp     | Yield (%) | $\lambda_{\text{max}}^{\text{EtOH}}$ (nm) | log $\epsilon$       | IR (cm <sup>-1</sup> ) |
|---------|--|--------|-----------|---|----------------------|------------------------|
| 1.      | C <sub>6</sub> H <sub>5</sub>  | 103-5° | 85        | 276                                       | 3.77                 |                        |
| 2.      | 4-CHO <sub>3</sub> C <sub>6</sub> H <sub>4</sub>                     | 87-9°  | 90        | 226<br>304-308                            | 4.16<br>3.40         | 1685 (s) 1718 1735     |
| 3.      | 3 : 4 (O·CH <sub>2</sub> ·O)C <sub>6</sub> H <sub>3</sub>            | 73-6°  | 100       | 226<br>290<br>300                         | 4.42<br>4.06<br>4.07 | 1680 1715 1733         |
| 4.      | 3 : 4 (OCH <sub>3</sub> ) <sub>2</sub> C <sub>6</sub> H <sub>3</sub> | 83°    | 95        | 226<br>286<br>312                         | 4.50<br>4.18<br>4.19 | 1860 (s) 1716 1735     |
| 5.      | 2ClC <sub>6</sub> H <sub>4</sub> *                                   |        | 70        | 268                                       | 3.92                 | 1682 1710-1730         |
| 6.      | 4ClC <sub>6</sub> H <sub>4</sub>                                     | 64-5°  | 80        | 214<br>286                                | 3.85<br>3.91         | 1705(s) 1730           |

\* This compound is viscous.

The reaction proceeded equally well with sodium ethoxide as the catalyst. Condensation with other aldehydes yielded the corresponding acids (Table I).



### Experimental

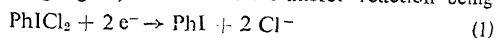
*Condensation of diethyl- $\gamma$ -oxo-pimelate and anisaldehyde in potassium tert.-butoxide.*—A mixture of diethyl- $\gamma$ -oxo-pimelate (5 g) and anisaldehyde (3.0 g) was added to potassium tert.-butoxide (from 1 g of potassium and 30 ml of tert.-butanol) and stirred for 45 minutes under inert anhydrous condition at room temperature. The reaction mixture was acidified with 6N hydrochloric acid and the tert.-butanol removed under reduced pressure. The residue was taken up in ether and the ethereal phase was repeatedly washed with ice-cold sodium carbonate solution. The alkaline phase on acidification gave  $\beta$ -arylidene- $\gamma$ -oxo- $\omega$ -carbethoxy hexanoic acid (6.3 g, 90%). m.p. 103-5°; Found: eq. wt., 317; C, 63.5; H, 6.4, reqd. for C<sub>16</sub>H<sub>20</sub>O<sub>6</sub>; eq. wt., 320; C, 63.75; H, 6.25%.

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### MEASUREMENT OF FORMAL POTENTIAL OF THE IODOBENZENE DICHLORIDE/IODOBENZENE COUPLE IN GLACIAL ACETIC ACID

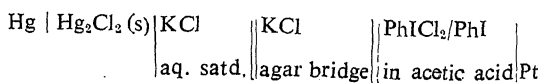
A SOLUTION of iodobenzene dichloride in dry acetic acid has been employed for the determination of a variety of reductants in non-aqueous and partially aqueous media<sup>1,2</sup>. In such media, iodobenzene dichloride (PhICl<sub>2</sub>) acts as a moderately strong oxidizing agent, the electron transfer reaction being



It is of practical importance to determine the redox potential of the couple. But the measurement of *standard* redox potential is difficult due to the involvement of various factors such as activity coefficients, liquid junction potentials, etc., and also since the medium is not aqueous. It is, however, possible to determine the *formal* potential. Lingane<sup>3</sup> also considers that the formal potential is a "kind of practical standard potential".

Iodobenzene (Koch-Light) was redistilled and used; iodobenzene dichloride was prepared as described earlier<sup>1</sup>. Glacial acetic acid was purified by the usual procedure. Standard solutions of both iodobenzene dichloride and iodobenzene in dry acetic acid were separately prepared; the strength of the former was checked by the iodometric method<sup>2</sup>. A Toshniwal CL 06—Titration Potentiometer with magic eye detector was used. A bright platinum strip was used as indicator electrode while an aqueous saturated calomel electrode (SCE) served as the reference one.

Mixed solutions of various compositions containing iodobenzene dichloride and iodobenzene were kept in a thermostat at 35  $\pm$  0.02° C. After ensuring the attainment of thermal equilibrium, the e.m.f. was determined for the following cell:



The formal potential ( $E^{\circ}_{\text{redox}}$ ) was then calculated using Nernst equation.

$$E_{\text{redox}} = E^{\circ}_{\text{redox}} + \frac{2.303 R T}{2 F} \log \frac{[\text{OX}]}{[\text{Red}]}$$

The values of  $E^{\circ}_{\text{redox}}$  are presented in Table I. As seen from the value of the formal potential (1.24 volts), iodobenzene dichloride in acetic acid is a moderately strong oxidizing agent.

TABLE I  
Measurement of the formal redox potential of the  
 $\text{PhICl}_2/\text{PhI}$  couple in acetic acid  
(Temperature =  $35 \pm 0.02^\circ \text{C}$ )

| Expt. No. | Mole ratio<br>$\text{PhICl}_2/\text{PhI}$ | E (volts) | $E^{\circ}$ (volts) |
|-----------|---|-----------|---------------------|
| 1         | 0.5880                                    | 1.234     | 1.241               |
| 2         | 1.176                                     | 1.244     | 1.242               |
| 3         | 1.764                                     | 1.252     | 1.244               |
| 4         | 0.8822                                    | 1.240     | 1.242               |
| 5         | 1.176                                     | 1.250     | 1.248               |
| 6         | 1.471                                     | 1.252     | 1.247               |

Dr. C. G. R. Nair (Department of Chemiistry, Kerala University) is thanked for helpful suggestions.  
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#### RAPID EXTRACTION AND DIRECT SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNT OF PALLADIUM WITH DIPHENYLCARBAZONE

DIPHENYLCARBAZONE forms chelate complexes with the ions of many heavy metals<sup>1</sup>. Its salt forming character with a number of metals was reported by Skinner and Ruheman<sup>2</sup>. Grosset<sup>3</sup> used it for detection of chromium. The reagent has also been used for the extractive spectrophotometric determination of mercury<sup>4,5</sup> and copper<sup>6</sup>. Its reaction with palladium is, however, not reported so far. It has been found that palladium in macro amounts forms a dark violet precipitate with diphenylcarbazone in acidic solution which is extractable with isobutyl methyl ketone. Extractive spectrophotometric determination of palladium in micro amounts using reagents, viz., dimethylglyoxime, dithiozone, *p*-nitroso-dimethylaniline, etc., is known. In this paper, we

describe the extractive spectrophotometric determination of palladium in micro amounts using diphenylcarbazone reagent.

#### Experimental

Absorbance measurements were made with a Beckmann DK-2 spectrophotometer. A stock solution was prepared by dissolving 1.1660 g of palladium chloride (B.D.H. Analar) in 100 ml of distilled water containing a few ml of concentrated HCl. The solutions of lower concentrations were prepared by dilution from the stock solution and was made 0.1 N with respect to nitric acid.

A 1% solution of diphenylcarbazone (B.D.H. product) in absolute alcohol was employed as the reagent.

#### Analytical and Separation Procedure

An aliquot of the palladium solution containing 1  $\mu\text{g}$  to 35  $\mu\text{g}$  of palladium was made upto 5 ml with 0.1 N nitric acid. Diphenylcarbazone reagent (1 ml) was added followed by the addition of 10 ml of isobutyl methyl ketone. The resulting mixtures were shaken for 1 minute and the organic layer was separated and allowed to stand for 40 minutes. The absorbance was measured at 610 nm against the pure solvent. The amounts of palladium in unknown solutions were calculated from the standard calibration curve. The results, which are the average of three determinations, in each case showed that the error involved is less than 2%. In the concentration range, 5–30  $\mu\text{g}$  of palladium, the error is less than 1%.

In a separate set of experiments, the standard palladium solution containing 21  $\mu\text{g}$  of palladium was mixed with an aqueous solution of the metal ions  $\text{Fe}^{3+}$ ,  $\text{Co}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pt}^{4+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Au}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{V}^{5+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Mo}^{6+}$ ,  $\text{W}^{6+}$  and  $\text{Mn}^{7+}$  in amounts almost equal to that of palladium taken and the volume in each was made upto 5 ml with 0.1 N nitric acid solution. The estimation was completed as described above. These experiments were repeated in presence of 100  $\mu\text{g}$  each of the above metal ions and also in presence of 100  $\mu\text{g}$  each of the common anions, viz.,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CN}^-$ ,  $\text{SCN}^-$ ,  $\text{C}_2\text{O}_4^{2-}$ ,  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$ . It was found that  $\text{Mo}^{6+}$ ,  $\text{V}^{5+}$  and  $\text{Fe}^{3+}$  and also  $\text{CN}^-$ ,  $\text{SCN}^-$  and  $\text{I}^-$  interfered badly and  $\text{Cr}^{6+}$  only slightly. The other ions did not interfere.

#### Discussion

Diphenylcarbazone forms a chelate with palladium in acidic solution which is extractable with isobutyl methyl ketone forming a blue violet solution. This complex has an absorption maximum at 610 nm and obeys Beer's law over the concentration range 0.1 to 3.5  $\mu\text{g}$  per ml. Measurement of absorbance immediately after extraction gave a low



value for the absorbance which, however, increased gradually with time and reached a constant value after 40 minutes and remained stable for more than 12 hours.

Although extraction of palladium was possible from dilute nitric acid (0.05 N to 0.5 N), the absorbance values of the extract decrease with an increasing acidity beyond 0.15 N. Maximum absorbance was obtained for the extract when the acid strength was 0.05 N to 0.15 N. The acid concentration in the aqueous layer was, therefore, maintained at 0.1 N.

A 1% alcoholic solution of the reagent was found to be most suited for the determination. When the reagent concentration exceeds 1%, there is significant absorption at 610 nm. This is not the case when the reagent concentration is  $\leq 1\%$ . Thus the absorbance values measured against the reagent blank do not differ from those measured against the pure solvent. Further the solution of the reagent in the solvent itself requires more than 24 hours time for the attainment of stability of the colour of the complex.

An examination of the effect of other ions revealed that iron (III), cobalt (III), nickel (II), platinum (IV), copper (II), silver (I), gold (III), zinc (II), cadmium (II), mercury (II), chromium (III), chromium (VI), molybdenum (VI), tungsten (VI), vanadium (V) and manganese (VII) when present in almost equal amounts with palladium did not interfere and a good recovery of palladium (within 4% error at  $\mu\text{g}$  level) was achieved. However, molybdenum (VI), vanadium (V), iron (III) and chromium (VI) when present in higher amounts interfered. Amongst the anions iodide, cyanide and thiocyanate interfered seriously when present even in very small amounts, whereas the chloride, bromide, fluoride, oxalate, sulphate and nitrate did not interfere even when present in excess.

Thus the method is simple and rapid and yet provides a good recovery of palladium in trace concentrations in presence of most of the common metal ions.

The authors express their sincere thanks to Professor N. N. Siddhanta and Dr. N. K. Baishya for their encouragement and help in carrying out this work.

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## TRANSFORMATION OF PLAGIOCLASE

A THIN pinkish aplite vein, varying from 3" to 6" in thickness, has been found cutting across both granite and dolerite at Ramapuram temple in the Mid-Pennar Reservoir project area in Anantapur District, Andhra Pradesh (Survey of India, Toposheet No. 57 F/5). The aplite is a compact vein in the granite but, as it enters the dolerite, changes its pattern into shreds and veinlets. As the dolerite is jointed, thin veinlets branching off from the vein, project themselves along with the joint planes of the dolerite.

Granite often contains two types of plagioclase of which one is the most commonly occurring, more basic oligoclase-andesine variety while the other is albite which generally occurs as rims and granules. The origin of such albite in granitic rocks has been discussed by earlier investigators (Rogers, 1961; Ramberg, 1962). Microscopic examination of the aplite has revealed evidence of decalcification of plagioclase and soda metasomatism with attendant development of albite.

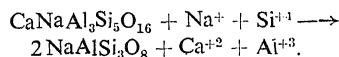
The aplite consists of micropegmatite (67.2%), quartz (6.8%), basic plagioclase (6.9%), albite (3.0%), epidote (5.4%), alteration materials (sericitic and clayey products) (8.0%), opaque ore (1.8%), and chlorite (0.9%). The most striking feature of the thin sections of this rock is the presence of a highly turbid, nearly opaque plagioclase surrounded by a mantle of fresh twinned albite [maximum extinction:  $16.5^\circ \perp (010)$ ] which in turn is partially surrounded by a chain of epidote grains (Figs. 1–3). These grains are variegated with tints in light green or light yellow. Some grains are feebly pleochroic while some are non-pleochroic.

The writer believes that the formation of epidote and albite rims must have taken the following course :

Most of the compounds of  $\text{Na}_2\text{O}-\text{CaO}-\text{SiO}_2$  are unstable and exist without dissociation only through a small range of temperature (Morey and Bowen, 1925). Earlier workers (Lyons, 1955; Fyfe *et al.*, 1958; Waard, 1959) have observed a gap in composition between albite and oligoclase. The more Ca-rich phase of the plagioclase is unstable in the presence of water and excess CaO, and the reaction :  $\text{Calcic plagioclase} \rightleftharpoons \text{albite} + \text{epidote}$ , is favoured

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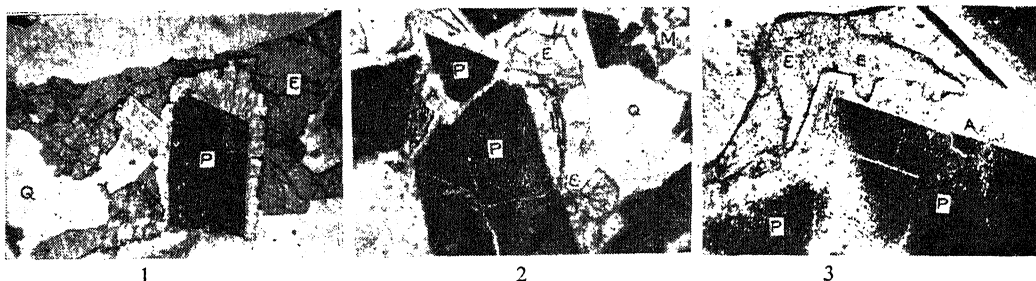
when the temperature reaches a point at which the velocity of the reaction attains a geologically finite rate (Deer *et al.*, 1963, p. 150). In this transformation, Ca and Al of plagioclase are released to enter the lattice of epidote.



It has been experimentally demonstrated (Eskola *et al.*, 1935) that in this reaction, the formation of albite from more basic plagioclase takes place without any appreciable change in volume which is evident in Figs. 1-3. Decalcification of more basic plagioclase and the consequent development of albite rim has also been suggested by Dietrich (1962) and Prasad (1968).

also due to Prof. M. G. C. Naidu for providing the facilities to carry out this work.

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FIGS. 1-3. Decalcification of more basic plagioclase into albite rim and epidote. P, More basic plagioclase; A, Albite; E, Epidote; Q, Quartz; M, Micropegmatite. Fig. 1. ( $\times 40$ ), Fig. 2 ( $\times 70$ ) and Fig. 3. ( $\times 100$ ). Infiltration of quartz and albite through cracks in the more basic plagioclase due to which the grain is also frayed in the upper plagioclase are emphasized by such infiltration.

The basic plagioclase also appears to be affected by soda metasomatism involving the fixation of Na and Si. Figures 2 and 3 reveal the infiltration of Na and Si, in the form of albite and quartz, through the cracks and cleavages in the more basic plagioclase.

Bowen (1928, p. 186) states that a slight decrease in the temperature, during the crystallisation of a plagioclase mixture, results in the reaction: Plagioclase + liquid = a little more plagioclase of somewhat more sodic composition. Vance (1965, p. 643) similarly argues that a superheated more albitic melt, by mixing with a cooler and more anorthitic material, could lead to partial resorption of plagioclase crystals in the latter and, on cooling, precipitation of some sodic plagioclase.

The observations made in this study are in accord with the statement that plagioclases in the range of composition  $\text{An}_{7-5}$  to  $\text{An}_{21-25}$  are sometimes divided into sodium-rich and calcium-rich regions (Deer *et al.*, 1963, p. 96 and pp. 104-105).

Grateful thanks are due to Dr. K. V. Suryanarayana for kindly going through the manuscript and offering valuable suggestions. Thanks are

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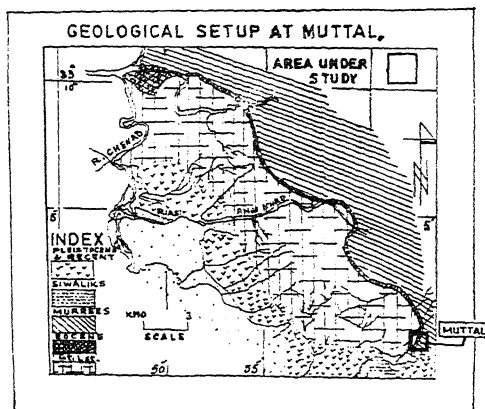
#### OCCURRENCE OF STROMATOLITES FROM THE GREAT LIMESTONE OF MUTTAL, UDHAMPUR DISTRICT, JAMMU PROVINCE, J AND K STATE, INDIA

THE present note records the occurrence of stromatolites from the dolomitic limestones of Muttal ( $32^{\circ} 59' \text{ N} : 75^{\circ} 02' \text{ E}$ ), at a place across the Dokhadda bridge. It lies on Survey of India Toposheet No. 43 P/1. It is connected by a 16 km long branch road from Tikri ( $32^{\circ} 56' 30'' \text{ N} : 74^{\circ} 57' \text{ E}$ ), about 45 km from Jammu on the National highway to Kashmir,

The Great Limestone in which the stromatolites occur is a part of the great Reasi inlier. The Great Limestone is a prominent formation in the outer Himalaya, occurring in the form of a chain of inliers, stretching from Purī ( $33^{\circ} 35' N : 73^{\circ} 55' E$ ) to Muttal ( $32^{\circ} 59' N : 75^{\circ} 02' E$ ), over a distance of about 120 km, along the prevalent strike of the outer Himalaya. The limestones are bluish grey in colour and frequently dolomitized with intercalations of cherty matter. The formation has been given different names from time to time. Medicott<sup>15</sup> (1876) and Simpson<sup>16</sup> (1904) called it the "Great Limestone", Wright<sup>22</sup> (1906) and Middlemiss<sup>8</sup> (1928) referred it as the "Sirban Limestone". Wadia<sup>21</sup> (1937) designated it as the "Jammu Limestone", while Sharma<sup>18</sup> (1970) called it as the "Vaishno Devi Dolostones".

Many have reported the occurrence of stromatolites in the Great Limestone. Among the pioneer workers, mention may, however, be made of Sharma (1970), Dasarathi<sup>12</sup> (1969), Gupta and Dixit<sup>13</sup> (1970, 1971, 1972), Valdiya<sup>19</sup> (1969), Raha (1972), Singh and Vimal<sup>17</sup> (1972) and Raha and Sastry<sup>13</sup> (1973). However, the stromatolites in the Great Limestone of Muttal remained unnoticed so far.

Regarding the regional geology, it may be pointed out that the Great Limestone forms the oldest group of rocks in the area, being overlain unconformably by the Eocene formations. The formation shows a strike of NW-SE with dips varying between  $45^{\circ}$  to  $70^{\circ}$  in the NE direction with minor warpings, flexurings and dislocations. The local variations both in the direction of dip and strike have also been observed (Map 1).



MAP 1

thickness of about 9 meters. The outcrop is separated from the parent rock at their strike ends, and the plane of separation contains limestone debris with shrubby vegetation acting as a cover.

Megascopically, the stromatolites are made up of calcic matter of variable character. The columns and cylinderoids are constituted of bluish grey limestone laminae. In the weathered portions, the laminae are distinct, isolated and crenulated. At places, cherty matter has also been observed. The cylinderoids expand upward from the base, the laminae are often domed towards the top and incurved around the edges. They are 4 to 6 cm in diameter at the base, expand upto 9 to 12 cm at the top, and have a height varying between 12 to 20 cm. No branching has been observed. Most of the columns are vertical but some of them are inclined. A few forms are ovoidal with a diameter of about 14 cm. They are generally formed by the concentration of mass around particular nuclei on the substratum.

The cylinderoidal forms have their length, far exceeding the width. The direction and the rate of growth are irregular. At places, the structure has arched lamella. Most of the cylinderoidal columns ramify "passively" or do not ramify at all. Such columnar forms (Fig. 1) belong to the collenia group and can be correlated with LLH-C structures of Logan *et al.*<sup>6</sup> (1964).



FIG. 1. The columnar stromatolites at Do-Khadd Muttal, Udhampur District, Jammu Province (J & K)

A few forms which are constricted at the bottom centre, and widening upwards, club-shaped with

The outcrop, exhibiting stromatolitic structures, is about 12 meters long, along the strike, with a

variable basal radii fit well in the generalised cryptozoön group (SH-V structures of Logan *et al.*, 1964).

The sections show massive or cryptocrystalline calcic matrix with veins of microcrystalline calcareous matter. The calcite grains are mostly anhedral or sub-hedral while the dolomite grains are typically rhomb-shaped. Some sections exhibit euhedral to sub-hedral grains of authigenic feldspar and quartz. The cryptocrystalline cherty matter is found in between the planes.

Regarding the genetic history of stromatolites, much controversy has arisen, but most of the workers, at least, agree on the point, that they are of algal origin, formed under marine conditions. They are formed in clear and shallow waters of well-protected basins.

The Great Limestone was regarded completely unfossiliferous and as such varied conjectures were made about its age. Medicott (1876) and Simpson (1904) regarded them of "Jurassic" age. Middlemiss assigned an "Infra-Trias" age, purely on the basis of their lithological traits. Lydekker<sup>10</sup> (Pascoe, 1964; p. 814) assigned a "Carboniferous" age, on the basis of doubtful presence of *Fenestella*. Wadia (1938) placed them between "Upper Carboniferous" and "Lower Permian" age, on the basis of their interbedded nature with supposed Agglomeratic Slates. Raina (1964) correlated these formations with the Krol and Shali carbonates of Himachal Pradesh, Raha and Sastry<sup>13</sup> (1973), on the basis of generic identification of stromatolites of Riasi limestones, assigned them "Middle to Upper Riphean" age. Since the Muttal limestone is a part of the Riasi inlier, they are identical in their age also.

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#### REGENERATION OF CRYPTORCHID TESTIS BY ORCHIDOPEXY IN ALBINO RATS

EXPERIMENTALLY induced cryptorchid testis (abdominal retention) is known to exhibit atrophic changes in the spermatogenic activity<sup>1,2</sup>. When gonadotropin or androgen therapy fails to restore normal spermatogenesis in cryptorchid testis, orchidopexy (scrotal restoration) is recommended<sup>3</sup>. The present investigation is to study the spermatogenic recuperation of the cryptorchid testis by orchidopexy qualitatively as well as quantitatively.

Bilateral cryptorchidism was performed by pushing the testis into the abdomen in adult rats of Holtzman strain, weighing 180 to 250 g (70-90 days old). After 15 days, orchidopexy was performed by pushing the abdominal testis into the scrotum as per the method of Nelson<sup>2</sup> with slight modifications. Suitable normal controls were maintained. They were autopsied 15 days after cryptorchidism or 60 days after orchidopexy. Testis and accessory organs were dissected, weighed, fixed in Bouin's fluid, sectioned and stained in PAS-Haematoxylin. Spermatogenic counts were expressed per 100 Sertoli cells as described by Dym and Clermont<sup>4</sup>.

Fifteen days after cryptorchidism, testis exhibits a significant reduction in weight by 64.6% ( $P < 0.001$ ) in relation to controls, wherein all tubules are devoid of spermatids and sperms, with persistent pachytene spermatocytes in 66.9% of tubules (Figs. 1 and 2), while all tubules exhibit type B spermatogonia. Such degenerated cryptorchid testis recovers partially by orchidopexy in 60 days wherein 81.4% tubules show normal spermatogenesis, while the rest are still degenerated.

## Cell Counts

As a result of destruction of germinal elements, the seminiferous tubules shrink resulting in the concentration of the remaining germ cells and Sertoli cells along the limiting membrane. The counts were therefore expressed per 100 Sertoli cells as a standard procedure for evaluation<sup>4</sup>. In cryptorchid testis, type B spermatogonia are con-

siderably reduced when compared to controls but the ratio between type B spermatogonia and pachytene spermatocytes is 1 : 1.99, while in the controls it is 1 : 2.1, indicating that though the number is reduced, the ratio is maintained almost the same (Table I). Sixty days after orchidopexy, the testis recuperates considerably (Fig. 3), wherein the number of spermatogonia, pachytene spermatocytes,

TABLE I

*Effect of orchidopexy on cryptorchid testes and accessory organs*

| Treatment                        | Organ wt. (mg)/100 gm body wt. $M \pm S.E.$ |                 |                 |                 |                  | Cell counts/100 Sertoli cells $M \pm S.E.$ |                            |                 |                             |
|----------------------------------|---|-----------------|-----------------|-----------------|------------------|--|----------------------------|-----------------|-----------------------------|
|                                  | Testes                                      | % reduction     | Epididymis      | Seminal vesicle | Ventral prostate | Type B spermatogonia                       | Pachytene spermatocytes    | Spermatis       | % tubules showing spermatid |
| Normal Controls                  | (5) 1416<br>$\pm 17$                        | ..              | 397<br>$\pm 14$ | 283<br>$\pm 16$ | 92<br>$\pm 12$   | 81<br>$\pm 11$                             | 174<br>$\pm 21$            | 750<br>$\pm 81$ | 100                         |
| Bilateral Cryptorchidism 15 days | (5) 502<br>$\pm 27$                         | 65 <sup>†</sup> | 251<br>$\pm 12$ | 392<br>$\pm 38$ | 113<br>$\pm 14$  | 44*<br>$\pm 9$                             | 87 <sup>†</sup><br>$\pm 8$ | $\pm$           | —                           |
| Bilateral Orchidopexy 60 days    | (5) 669<br>$\pm 58$                         | 53 <sup>†</sup> | 255<br>$\pm 14$ | 324<br>$\pm 34$ | 123<br>$\pm 17$  | 72<br>$\pm 14$                             | 282<br>$\pm 31$            | 732<br>$\pm 77$ | 81                          |

$M \pm S.E.$  = Arithmetic Mean  $\pm$  Standard Error.

Number in parenthesis denotes the number of rats.

Probability in relation to controls P \* 0.02 <sup>†</sup> 0.001.



FIGS. 1-3. Fig. 1. T.S. of the testis of control rat showing normal spermatogenesis,  $\times 270$ . Fig. 2. T.S. of the testis after 15 days of cryptorchidism exhibiting the shrinkage of tubules with degenerated seminiferous epithelium devoid of spermatids and sperms,  $\times 270$ . Fig. 3. T.S. of the testis after 60 days of orchidopexy showing recuperated tubule with normal spermatogenesis and the other still exhibits degeneration without spermatids and sperms,  $\times 270$ .

(Ps, Pachytene spermatocytes; Se, Sertoli cells; Sg, Spermatogonia; Sp, Spermatids.)

spermatids and sperms tend towards normalcy in terms of cell counts per 100 Sertoli cells (Table I).

Moore<sup>1</sup> and Nelson<sup>2</sup> attribute the atrophic changes in seminiferous epithelium to be due to cryptorchidism, to increased abdominal temperature as compared to that of the scrotum. Chowdhury and Steinberger<sup>3</sup> have shown that spermatids and sperms are most susceptible to heat. It is seen in this experiment that sperms and spermatids degenerated while spermatogonia and pachytene spermatocyte persist after cryptorchidism. On orchidopexy, the cell types recuperate to normalcy, with the cell counts similar to that of controls (Table I). No significant change is discernible in the weights of seminal vesicle and ventral prostate, indicating that the androgenic production is not hampered. However, the epididymal weight is significantly reduced in cryptorchid animals ( $P < 0.001$ ). According to Amatayakul *et al.*<sup>6</sup>, the serum levels of testosterone are reduced considerably after cryptorchidism which may cause degeneration of epididymis, as its physiological integrity depends on testosterone as stated by Rajalakshmi and Prasad<sup>7</sup>. The reduction in epididymal weight is alluded to the reduced biosynthesis of testosterone, possibly due to partial defect in enzyme system, resulting in the increased production of androstenedione and dehydroepiandrosterone which maintains the accessory organs as hypothesized by Amatayakul *et al.*<sup>6</sup>. Further experiments are in progress to hasten the rate of recuperation of degenerated testis.

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# IRRADIATION-INDUCED STERILITY IN THE MALES AND FEMALES OF *CALLOSBRUCHUS* *ANALIS* (F.) (BRUCHIDAE: COLEOPTERA)

THE standardization of the doses of radiation required for sterilizing a few pests of stored grains has already been worked out<sup>1,2</sup>. Such information in respect of a few others is still wanted. The present report deals with the production of sterile males and females of *Callosobruchus analis* by irradiating its pupae of different ages under a cobalt<sup>60</sup> source fitted in the Irradiation Laboratory, P.G.I., Chandigarh.

The immature stages of *C. analis* required for these investigations were bred on the seeds of *Phaseolus aureus* (Moong) at 30° C and R.H. 70%. The pupal stage under these conditions lasts for five days. Different batches of pupae (one, two, three and four-day old) were irradiated separately with four doses of 1,000, 1,500, 2,000 and 2,500 rads. The adult males and the females that emerged from the treated pupae were allowed to copulate with the normal adults of the opposite sex. The fate of the eggs laid by the copulated females in each combination was watched. The mortality of treated pupae under different conditions was also recorded. The particular set of treatment, which showed the minimum mortality of pupae and produced eggs which failed to hatch, was considered to be the best.

The irradiation of one-day old pupae with all the four doses did not produce useful results, because the mortality of eggs was rather low in all the cases, with the maximum of 71.5% recorded with a dose of 1,000 rads. The two-day old pupae, when irradiated with 1,500, 2,000 and 2,500 rads, showed more than 50% pupal mortality and the resulting adults were greatly damaged, almost incapable of copulation. With a dose of 1,000 rads, on the other hand, the mortality in the treated pupae is low, i.e., 12.5% and the sterile males retain the normal vigour for copulation and the eggs produced by the females mated by them showed 100% mortality. Apparently, the desired type of mortality in males can be produced by irradiating two-day old pupae with a dose of 1,000 rads. Under the same conditions, the sterility induced in females is, however, very low as only 44.4% of the eggs failed to hatch. In the three-day old pupae, higher doses of 2,000 and 2,500 rads produced a very high mortality in the pupae. With the two lower doses, the sterility in the males is only 72% in both the cases while a 100% sterility is induced in females with a dose of 1,500 rads although the mortality of pupae in this treatment is also slightly high at 39%. The four-day old pupae behave in a manner similar to the three-day old pupae as they also

undergo a very high mortality with higher doses of 2,000 and 2,500 rads. The lower two doses give fairly good results, with low pupal mortality, but fail to give 100% sterility in either sex.

It is thus evident from the above that the desired type of sterile males and sterile females of *C. analis*, suitable for control operations, can be produced by irradiating two-day old pupae by 1,000 rads and three-day old pupae by 1,500 rads respectively. In the allied bruchid, *C. chinensis*<sup>3</sup>, the adult insects were exposed to a high dose of 42,000 rads to produce sterile males and females. In a few other pests of stored grains the adults were exposed to doses varying from 6,000 to 16,000 rads for this purpose<sup>4,5</sup>.

The author is grateful to Dr. G. P. Sharma, Head of the Department, for providing facilities and to the authorities of P.G.I., Chandigarh, for permission to make use of cobalt<sup>60</sup> unit.

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## A NOTE ON THE OCCURRENCE OF PROTEIN COMPLEXES IN THE HAEMOLYMPH OF CRABS

HAEMOCYANIN multiplicity is well known in many crustaceans<sup>1-6</sup>, but previous works do not record the occurrence of complexes of haemocyanin fractions with other proteins. Only in *Carcinus maenas* a single glycolipoprotein complex is reported<sup>7</sup>, but the present study on two crabs shows that a number of haemolymph proteins are possibly linked.

The haemolymph proteins in intermoult males of *Scylla serrata* (aquatic) and *Cardisoma carnifex* (terrestrial) were fractionated by 7% polyacrylamide gel electrophoresis. The gels were stained for haemocyanins, lipoproteins and glycoproteins using O-dianisidine-peroxidase<sup>1</sup>, oil red O and periodic acid Schiff (PAS) reaction respectively.

The haemolymph of *Scylla serrata* (Fig. 1) showed five fast moving haemocyanin fractions of which all excepting the fastest one were positive to oil red O and PAS. This seems to indicate that possibly the haemocyanin fractions are linked with lipo and glycoproteins. Out of the total of 11 lipo and 8 glycoprotein fractions, evident in the haemolymph of *S. serrata*, eight glycoprotein fractions are identical in mobility with lipoprotein fractions which is indicative of their close bondage. Three of the lipoprotein fractions are however free. It appears, therefore, that there are four haemocyanin lipoglycoprotein complexes and eight lipo-glycoprotein complexes in this crab.

In *Cardisoma carnifex* (Fig. 1), only two haemocyanin fractions, one fast and another slow moving, could be discerned. There are 8 glycoprotein fractions and 5 lipoprotein fractions in the haemolymph of this crab. The two haemocyanin fractions are

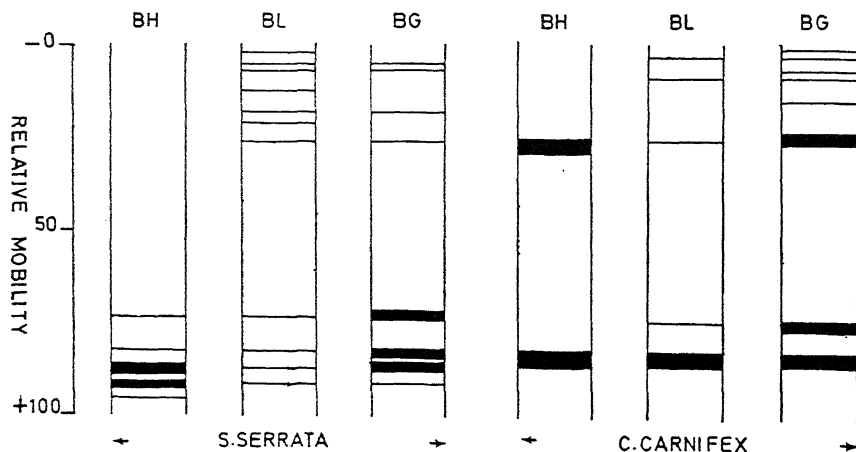


FIG. 1. Diagrammatic representation of haemolymph proteins of *Scylla serrata* and *Cardisoma carnifex*. BH, Haemocyanin. BL, Lipoprotein. BG, Glycoprotein.

identical in mobility with two of the lipoproteins and glycoproteins. All the five lipoproteins are identical in mobility with five of the eight glycoproteins, leaving three glycoproteins free. In other words, in the haemolymph of *C. carnifex*, two haemocyanin, lipo-glycoprotein complexes and five lipo-glycoprotein complexes are evident.

No information is yet available on bonding of haemocyanin with other haemolymph proteins. The present study, however, indicates the possible complexes of the haemocyanins with lipo and glycoproteins. Obviously a number of lipo and glycoprotein complexes also seem to exist in the haemolymph of crabs. It is very interesting to note here that while all glycoproteins appear to be complexed, only some of the lipoproteins are free in the aquatic crab *S. serrata* whereas in the terrestrial crab, *C. carnifex*, all the lipoproteins appear to be complexed but some of the glycoproteins seem to be free. It thus seems likely that the ecological factors may have an influence on the nature of haemolymph protein complexes in crabs.

The occurrence of greater number of haemocyanin fractions in *S. serrata* follows the fact that this crab is aquatic where more copper for the synthesis of haemocyanin is available. It is likely that, the haemocyanins perhaps serve also as carrier molecules for the lipo and glycoproteins. Further studies on crabs from many ecological niches may throw more light on this problem.

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# CALCINOSIS IN A FOWL (*GALLUS DOMESTICUS*)

THE present communication pertains to generalized calcification encountered in a fowl out of 13,710 birds necropsied in this department during the last 3½ years. Keeping in view the rarity of this disease in poultry, as evidenced by the scanty information in the available literature, it is considered worthwhile to record this condition.

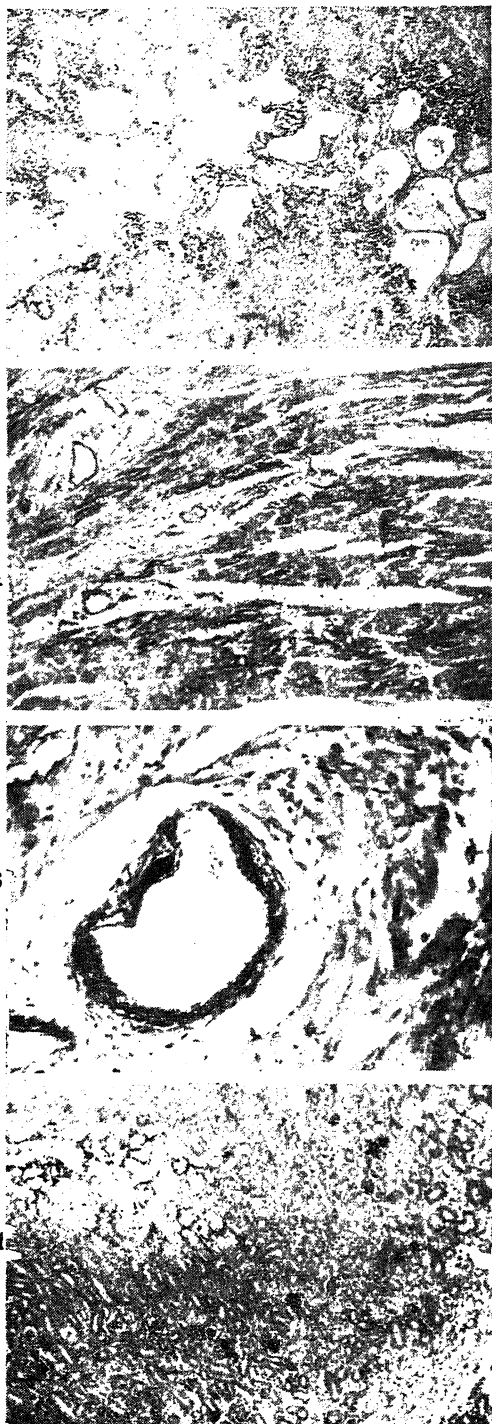
Grossly, whitish foci on the myocardium and numerous minute nodular growths scattered throughout the lung parenchyma were the predominant features. On cutting, the nodules produced a grating sound indicative of mineralization. The myocardium was hard in consistency and revealed greyish white discolouration. Lungs, kidneys, liver, adrenal glands and ovary presented greyish white appearance. The extensive deposition of urate crystals in the renal tubules resulted in their unusual prominence.

The heart blood was collected aseptically for cultural examination and the relevant material was fixed in 10% formalin for histopathology. The tissues were processed through routine paraffin embedding technique, cut at 5 micron thickness and were stained with haematoxylin and eosin (HE). For the demonstration of calcium salts and acid mucopolysaccharides von Kossa and Periodic Acid Schiff (PAS) staining methods were employed.

On microscopic examination varying degree of calcification was observed affecting lungs, kidneys, myocardium, adrenals, coeliac ganglion, ovary liver, cerebrum and trachea. The lungs, myocardium and kidneys were most severely involved. In the HE and von Kossa stained sections, the lungs were seen studded with fine basophilic granular deposits distributed irregularly along the respiratory tubules and around the smooth muscle cells of the tertiary bronchi (Fig. 1). Lymphoid aggregates were observed in the submucosa of the secondary bronchi along with widespread haemorrhages. Calcium deposition affected all the blood vessels and muscles of the myocardium (Fig. 2) and severely affected walls of the small veins which showed metaplastic changes evidenced by the formation of osteoid cells (Fig. 3). Lymphocytic infiltration was noticed in the pericardium. The kidneys revealed rather advanced degree of calcification replacing at several places, the glomeruli and renal tubules (Fig. 4). The metaplastic changes leading to osteoid cell formation were noticed in the severely calcified areas in the interstitial renal tissue. The mineralization in the form of linear streaks resulted in thickening of the tubular basement membrane. The epithelial lining of the tubules showed degenerative changes along with desquamation and haemorrhages at some foci. The interstitial tissue of adrenal glands

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FIGS. 1-4. Fig. 1. Lung: Calcium deposition along the respiratory tubules and around the smooth muscle cells of the tertiary bronchi. He,  $\times 30$ . Fig. 2. Myocardium: Calcification affecting the

blood vessels and muscles. HE,  $\times 30$ . Fig. 3. Myocardium: Osteoid cell formation in the severely calcified wall of the coronary vein. HE,  $\times 70$ . Fig. 4. Kidney: Advanced calcification involving walls of the tubules and interstitial space. HE,  $\times 30$ .

also revealed calcium deposition. Moderate type of mineralization affected blood vessels of the ovary, whereas, calcification was more obvious in the coeliac ganglion replacing thereby the ganglion nerve fibres. The matrix of tracheal cartilages had undergone calcification. Laying down of calcium salts was of moderate intensity in the hepatic parenchyma as well as in the blood vessel walls. There was lymphocytic infiltration in the perivascular zones that replaced hepatic parenchyma at isolated areas. Minute aggregates of fine calcific granules were observed in the cerebrum around the small blood vessels when stained with von Kossa. The neurons showed degenerative changes leading to satellitosis and neuronophagia. Aorta, intestine, bursa fabricius and peripheral nerves failed to reveal the calcium deposits. No organisms of pathological importance could be isolated from the heart blood.

The generalized calcification affecting majority of parenchymatous organs as noticed in the present case could not be traced in the available literature, however, Gill *et al.* (1972) have reported such lesions in some of the organs. Lesions of calcinosis observed in lungs during this investigation were almost identical with the observations of Parihar and Singh (1971) except the appearance of giant cells which were not observed during this study. The alterations met with in the kidneys were much advanced than those described by Marcado and Brand (1964) and Gill *et al.* (*loc. cit.*). The acid mucopolysaccharides were not demonstrable in the calcified areas as reported by Gill *et al.* (*loc. cit.*). The etiology of this ailment remained uncertain, however, extensive mineralization in diverse situations might be attributed to the disturbed mineral metabolism.

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# HAEMOGLOBIN PALYMORPHISM IN GOATS

IN Indian goats while there is a report of occurrence of only one haemoglobin (Hb) type some workers<sup>2,3</sup> have reported 2 types of Hb. However, in some of the foreign breeds <sup>3+5-6</sup> and also 4<sup>7</sup> Hb types have been established.

The study referred here is related to 76 Barbari and 70 Jamnapari goats in which Hb polymorphism was studied using horizontal paper electrophoresis in Tris-borate<sup>8</sup> buffer at a constant voltage of 200 volts for 18 hours.

In both the Barbari and Jamnapari goats 3 polymorphic Hb types, viz., homozygous Hb A and Hb B and heterozygous Hb AB as shown in the figure were found to occur. Hb A type was preponderant in both the breeds being 89.5 and 90.0% in Barbari and Jamnapari respectively. The distribution of Hb B was 2.64% and 1.43% and of Hb AB 7.89% and 8.57% in Barbari and Jamnapari breeds respectively.

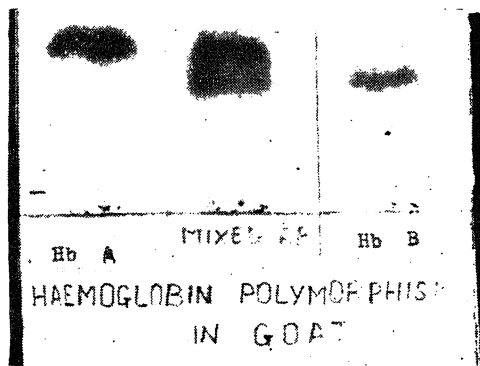


FIG. 1

The observation of the presence of homozygous Hb B type in this study is of significance since it has not been reported as yet by workers in Indian goats. Giri and Pillai<sup>1</sup> observed only Hb A type while Khanalkar *et al.*<sup>2</sup> and Balani *et al.*<sup>3</sup> have reported Hb A and Hb AB types.

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## SEX IDENTIFICATION OF *SPODOPTERA* (*PRODENIA*) *LITURA* (FABRICIUS) BY WING COLOURATION

DURING investigations at Jabalpur the male and female moths of *S. litura* revealed a common pattern of wing venation which was similar to the C and C<sub>1</sub> figures of Bhattacharjee and Raghavan (1967), but the wing colouration was very distinct in both the sexes (Fig. 1) and the separation of male from the female was rendered easy.



FIG. 1. Colour pattern of fore-wings—male and female moths of *Spodoptera litura*.

The male of *S. litura* possesses a prominent large wide yellow oblique patch (a) in the centre of the fore-wing, which extended from the costal margin to the cubitus below, while the inner margin of the wing was yellowish for the most part. In addition, a small triangular yellow patch (b) between the subcostal (S<sub>c</sub>) and radial (R<sub>1</sub>) veins, and another oblique yellow patch (c) reaching the apex near the outer margin of the fore-wing were noted. On the other hand, the central oblique yellow patch in the fore-wing of the female was marked with brown scales with discontinuous patch unlike the male. It was also distinctly crossed like an arrow a yellow median vein. Thus the colour pattern of the fore-wings served as an important criterion in sex identification of *S. litura*.

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### STUDIES ON CONTROL OF BLACKSPOT FORMATION ON MANGOES

DURING our studies on the storage and ripening of mangoes, we came across the development of blackspots on the surface of Alphonso mango. The mold *Rhizoctonia bataticola* was found to be responsible

5 min) and kept for ripening, the normal colour, appearance, taste and flavour were maintained indicating that these compounds do not affect the organoleptic characteristics of the fruit and hence could be employed successfully for the preservation of mangoes.

TABLE I  
Effect of fungicides on the growth of *R. bataticola*

| Concentration<br>( $\mu$ g)/<br>100 ml<br>flask | Percentage inhibition |            |       |                       |       |                       |                      |              |
|---|-----------------------|------------|-------|-----------------------|-------|-----------------------|----------------------|--------------|
|   | Aureo-<br>fungin      | Boric acid | Borax | Copper<br>oxychloride | Zineb | Calcium<br>propionate | Potassium<br>sorbate | Vitamin<br>K |
| 250   | 22                    | 29         | 71    | 5                     | 17    | —                     | —                    | —            |
| 500   | 93                    | 34         | 100   | 19                    | 26    | 56                    | 25                   | 96           |
| 1000  | 100                   | 47         | 100   | 29                    | 37    | 87                    | 43                   | 98           |
| 2000  | —                     | 74         | 100   | 60                    | 77    | 93                    | 52                   | —            |
| 2500  | 100                   | —          | —     | —                     | —     | —                     | —                    | 100          |
| 4000  | —                     | 76         | 100   | 63                    | 100   | —                     | —                    | —            |
| 5000  | —                     | —          | —     | 100                   | 100   | 97                    | 93                   | 100          |

— Not determined.

for about 40% spoilage due to blackspots on mango<sup>1</sup>. Earlier we had reported about the biochemical changes involved during the development of blackspots on mango<sup>1</sup>. Present communication deals with the study of certain antifungal compounds which inhibit the growth of the mold *Rhizoctonia bataticola* and reduce the spoilage. These compounds can be used successfully for the preservation of the mangoes.

The mold was grown in the synthetic medium of the following composition (in grams): Glucose 4,  $\text{NH}_4\text{Cl}$  0.5,  $\text{NH}_4\text{NO}_3$  0.1,  $\text{Na}_2\text{SO}_4$  0.02,  $\text{K}_2\text{HPO}_4$  0.1,  $\text{MgSO}_4$  0.01, Water 1000 ml, pH 5.5. The incubation was carried out at 30° C for 7 days. Mycelia were collected after filtration, dried and weighed. Mold was grown with and without antifungal compounds along with controls.

The results in Table I indicate that aureofungin, boric acid, borax, copper oxychloride, zineb, calcium propionate, potassium sorbate and vitamin K inhibited the growth of the mold. Vitamin K, aureofungin and borax were found to be the most effective. Beccari (1969)<sup>2</sup> had reported the successful use of vitamin K for the preservation of bananas and inhibition of the growth of the mold *Gleosporeum musarum* and *Fusarium* sp. Aureofungin has been used with success in controlling post-harvest fruit rot<sup>3,4,5</sup> and has been used for the preservation of tomatoes, bananas, oranges and figs.

When unripe mangoes were dip-treated in the solutions of aureofungin (10 mg % for 2 min), Vitamin K (0.1% for 2 min) and borax (2% for

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### EFFECT OF ACTINOMYCIN-D ON THE INCORPORATION OF URIDINE <sup>3</sup>H AND ON RNA SYNTHESIS IN HEALTHY AND CHLOROTIC MOTTLE VIRUS INFECTED LEAVES OF COWPEA

A good deal of information is available on the site and nature of synthesis of tobacco mosaic virus (TMV) through microspectrophotometric (Zech and Vogt-Kohne, 1955), immunofluorescence (Hirai and Hirai, 1964; Shalla and Amici, 1967) and autoradiographic studies (Smith and Schlegel, 1964; Langenberg and Schlegel, 1967; deZoeten and Schlegel, 1967). Smith and Schlegel (1964) further reported that actinomycin-D (AMD), when incorporated into a host-virus system, inhibits the synthesis of host RNA but not the viral RNA. All these

informations were available with TMV, which is a rod-shaped virus. However, no such information is available with regard to spherical viruses. The present study was undertaken with a view to obtaining informations on the site of synthesis and localisation of RNA of some spherical virus.

#### Materials and Experimental Procedure

Cowpea chlorotic mottle virus (CCMV), a small spherical virus measuring 32 nm in diameter (Kuhn, 1964) was selected. Cowpea (*Vigna sinensis*) cv. Early Ramshorn, raised from disease free stock, was used as test plants. At 2-3 trifoliate leaf stage, the plants were inoculated with highly infectious sap from cowpea leaves diluted to 1 : 5 with 0.01 M neutral phosphate buffer containing 10% Celite. The plants were then put inside a growth chamber at 30° C with 16 hr photoperiod and an illumination of 850 ft. candles.

Four days after inoculation, samples were collected from the test plants. For each treatment 3 mm discs were punched out at random from younger leaves. Actinomycin D (AMD) @ 100.00 µmg/ml was vacuum infiltrated for 1 hr into the samples. After infiltration, the discs were dried on filter papers moistened with AMD or distilled water, as the case may be. The discs were then floated on solutions of tritiated uridine (uridine <sup>3</sup>H @ 25 mCi/ml) for 2 hr for incorporation. They were then washed under running water for 3 hr to remove all labelled uridine which was not incorporated, and fixed at 4% glutaraldehyde in 0.05 M neutral phosphate buffer for 1 hr. The fixed tissues were washed in 3 changes of buffer for 45 min, fixed in 1% Osmium tetroxide for 2 hr and again washed in 3 changes of buffer.

The fixed material was then dehydrated through a series of alcohol (30%, 50%, 70%, 85%, 95% and 100%), 2 changes of propylene oxide, infiltrated with propylene oxide and resin mixture (1 : 1) for 2 hr and then embedded in resin mixture and kept overnight at 60° C. BEEM capsules were used for making blocks. Sections of 0.5-1.0 µm thickness were cut in a Porterblum ultra microtome using glass knives.

For autoradiography, the sections were coated with a thin layer of Ilford 11-4 emulsion and then kept for exposure in a light tight box containing silica gel for 15 days. After exposure, the autoradiographs were developed in Kodak D-19 developer for 3 min, washed, dried, mounted and examined under a phase contrast microscope.

#### Results and Discussion

It was observed that uridine <sup>3</sup>H was highly incorporated in the nuclei and cytoplasm of the AMD treated leaf discs infected with CCMV but

it was much less so in treated healthy discs. No considerable difference in incorporation of uridine <sup>3</sup>H as evidenced by labelling was observed in sections from healthy and infected non-treated leaves. Similar observations were made by Smith and Schlegel (1964) in case of TMV who also reported incorporation of uridine <sup>3</sup>H in nucleus in general and nucleolus in particular.

The results indicate some specific functions of nucleus and cytoplasm in uridine metabolism of cells infected with CCMV. The results also indicate that AMD inhibits the incorporation of uridine in healthy tissues thereby inhibiting synthesis of host RNA but in cells where multiplication of CCMV takes place, it does not interfere with the incorporation. It is probable that nucleus and cytoplasm might have taken part in some steps in the synthesis of the viral RNA of the CCMV. This study was aimed at perfecting the techniques. Further studies are in progress.

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#### A NOTE ON THE STOMATAL ONTOGENY AND SYSTEMATIC POSITION OF *ABRUS* *PRECATORIUS* L.

IN their recent publication<sup>1</sup> Venkateshwarlu and Seshavatham have discussed the systematic position of *Abrus* L. in the Fabaceae (Papilionaceae) and suggested that this genus should be removed from the tribe Viciae and included in the tribe Phaseoleae on the basis of the evidence obtained from embryological and other studies. As there is

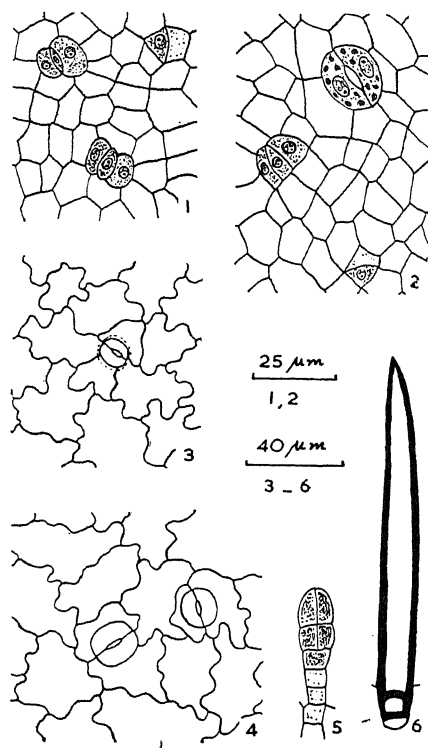
no report on the stomatogenesis of *Abrus precatorius* L. the present work is taken up to find out the systematic position of the genus.

Leaves are hypostomatic, possessing the stomata of aperigenous, anomo-, tetra-, aniso-mesoperigenous and para-mesogenous types<sup>2</sup>. Of them anisocytic stomata are more frequent while tetracytic type is less in number. The stomata are oriented in various directions and differentiate in a mixed sequence.

Any protodermal cell undergoes an unequal division to give rise to a smaller meristemoid and a larger sister cell which becomes vacuolated and simulates other protodermal cells soon. The meristemoids are triangular or trapezoidal in shape with denser cytoplasm and distinct nucleus (Figs. 1, 2). These are mostly dolabrate, producing anomo-, tetra-, aniso-mesoperigenous and para-mesogenous stomata. Rarely do they directly act as GMC (guard cell mother cell). It divides vertically to produce two guard cells of equal size. This is termed aperigenous type<sup>2</sup>. Interestingly these stomata differentiate very early when the other stomatal types are in developmental stages (Fig. 2). Perigenous mode of origin of the above stoma is evidenced by its larger size in contrast to the small size of the encircling neighbouring cells, as it has been observed in the leaves of *Clerodendrum phlomidis*.<sup>3</sup>

Tetra-, aniso-mesoperigenous and para-mesogenous stomata are derived from the dolabrate meristemoids depending upon the placement of its second wall. A similar mode of ontogeny for the above stomatal types has already been reported in *Zornia*<sup>4</sup>. As a result of the first division of the meristemoid, one larger mesogenous subsidiary cell and a smaller cell are produced. Of these two, the latter puts forth a vertical wall with the result that a GMC and a second mesogenous subsidiary cell are formed. Frequently (37%) aniso-mesoperigenous stomata are derived when the above second wall intersects the first one at only one point. Consequently a conical GMC is flanked by two lateral subsidiaries on three sides and by a perigenous cell on the fourth side (Figs. 2, 4). Less frequently (28%) para-mesogenous type develops when the second wall intersects the former at both the poles. Here, a lenticular GMC is completely encircled by two lateral subsidiaries of mesogenous origin (Figs. 1, 4). Occasionally (15%) the second wall is laid parallel to the previous one so as to produce a tetra-mesoperigenous stoma (Fig. 1). In this case a band-shaped GMC is flanked by two mesogenous subsidiaries on the lateral sides and by two perigenous ones on the polar ends. Infrequently one or two subsidiaries of anisocytic stomata divide radially resulting in the

anomo-mesoperigenous stomata with 4-5 neighbouring cells (Fig. 3).



FIGS. 1-6. *Abrus precatorius*. Fig. 1. para-mesogenous and tetra-mesoperigenous stomata in developmental stage. Fig. 2. Aperigenous stoma and dolabrate anisocytic stoma. Fig. 3. Anomo-mesoperigenous stoma. Fig. 4. Para-mesogenous and aniso-mesoperigenous stomata. Fig. 5. Short, clavate glandular hair. Fig. 6. Uniseriate papilionaceous non-glandular hair.

Metcalf and Chalk<sup>5</sup> stated that paracytic and anomocytic stomata are common in the Phaseoleae and the Viciae respectively though some stomata in *Cicer* and *Vicia* are paracytic. *Pisum sativum* has been shown to possess aniso-mesoperigenous stomata<sup>6</sup> while those of *Vicia faba* are aperigenous (anomocytic)<sup>7</sup>. However our studies and those of Paliwal and his co-workers<sup>7</sup> do not reveal the occurrence of paracytic stomata in *Cicer arietinum* and *Vicia faba* as reported earlier<sup>5</sup>. Thus it is clear that paracytic type is absent in the Viciae. On the contrary, leaves of *Abrus precatorius* show para-mesogenous stomata in addition to the other types as those of Phaseoleae<sup>8-9,10</sup>. Besides, short, clavate glandular hairs (Fig. 5), reported to occur in the Phaseoleae, were seen in the leaf margin of *A. precatorius* along with the typical papilionaceous, uniseriate, non-glandular hairs (Fig. 6). Evidently,

*A. precatorius* bears close resemblances to those of Phaseoleae in possessing para-mesogenous stomata and short clavate glandular hairs. Hence the present study on the stomatogenesis and the hairs substantiates the contention of Venkateshwarlu and Seshavatham and other previous workers<sup>1</sup> and suggests the removal of the genus *Abrus* from the Viciae and its inclusion in the Phaseoleae.

The author expresses his deep gratitude to Professor S. L. Basu, for his kind encouragement.

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#### INDUCED TETRAPLOIDY IN CURVED SPINE MUTANT OF *SOLANUM KHASIANUM* CIARKE

THE importance of *Solanum khasianum* for its high solasodine content has been recognised by several workers<sup>1-3</sup>. However, its large-scale cultivation by pharmaceutical industries, manufacturing steroid hormones, is restricted because of the occurrence of spines on the plant parts.

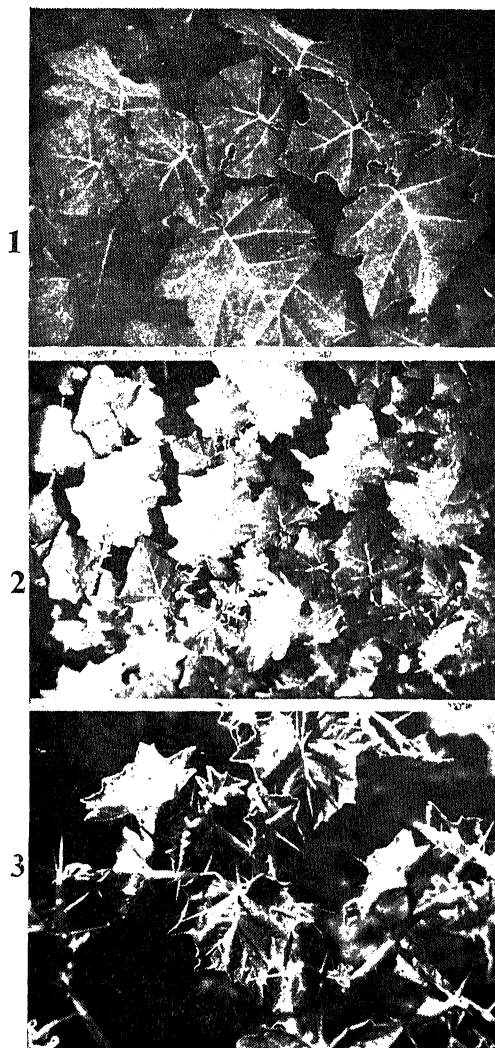
Exploitation of this medicinal plant would be more economical, if a variety having less spines and higher solasodine content is developed. Attempts were made in this direction by using mutagenic agents such as colchicine and gamma-rays.

Isolation of a mutant, which had thick curved and blunt spines, in  $\gamma$ -irradiated population was reported earlier<sup>4</sup>. This curved spine mutant was used for obtaining tetraploids by colchicine. Seedlings (100) were treated by 0.1% colchicine as described by Janaki Ammal and Bhatt<sup>5</sup>.

Cytological studies of leaf tip cells revealed 25% of the treated plants to be initially tetraploids. However all of them excepting two proved to be mixoploids. These two  $C_1$  plants maintained tetraploidy ( $4n = 48$ ) as evidenced from PMC studies. They possessed thick, dark leaves, bigger flowers than the control and bigger pollen grains which

were 25% fertile. Fruits (10), obtained from these two tetraploids, had poor seed setting, and low germinability of these seeds gave only five  $C_2$  plants.

Variation in the number of spines per leaf—1-5 compared to 5-10 in the control—was noticed in  $C_2$  and  $C_3$  generations of mutant tetraploids (Figs. 1, 2 and 3). Similar variations were observed in the tetraploids obtained from unirradiated controls<sup>5</sup>. It may be emphasised that the spineless tetraploids did not bear flowers. Some of them with the reduced the number of spines had a few flowers which did not set fruits. These observations suggested a probable association of spinelessness with sterility, since spiny tetraploids were reasonably fertile.



FIGS. 1-3. Fig. 1. Control. Fig. 2: Mutant. Fig. 3. Tetraploid mutant.

TABLE I  
Improvement in fertility of curved spine mutant tetraploids

| Generation of tetraploidy     | No. of plants | Pollen fertility % | Av. No. of fruits per plant | Av. No. of seeds per fruit | Germination % | Wt./fruit g |
|-------------------------------|---------------|--------------------|-----------------------------|----------------------------|---------------|-------------|
| Control (Diploid)             | 10            | 100                | 118                         | 220                        | 100           | 3.10        |
| Curved spine mutant (Diploid) | 17            | 100                | 186                         | 200                        | 90            | 3.41        |
| C <sub>1</sub>                | 2             | 25                 | 5                           | 2.5                        | 20            | 0.95        |
| C <sub>2</sub>                | 5             | 50                 | 10                          | 41.0                       | 35            | 1.45        |
| C <sub>3</sub>                | 25            | 70                 | 93                          | 75.0                       | 48            | 2.35        |

This suggestion got further support when a spineless mutant obtained in irradiated population produced no flowers. Another mutant which had very much reduced spines, produced a few flowers and very small fruits (0.95 g/fruit). Due to poor seed setting and germination, it was lost in C<sub>3</sub> generation (unpublished data)<sup>6</sup>.

Contrary to all the observations, mutant tetraploids with reduced number of spines, which were more curved than those in the diploid controls, have shown marked improvement in their fertility during C<sub>2</sub> and C<sub>3</sub> generations. Table I shows that pollen fertility increased from 25% to 70% while fruit setting increased from 5 to 93. The number of seeds per fruit also showed remarkable increase from 2.5 to 75, so also germinability of these seeds. Similar trend, evidenced in fruit size and weight, indicates that further efforts in improving and selecting a suitable tetraploid mutant may be rewarding.

The fruits of mutant and tetraploids are being analysed for their solasodine contents, while C<sub>4</sub> generation studies are in progress.

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#### EFFECT OF RED AND FAR RED ILLUMINATIONS ON THE GERMINATION OF SPORES OF TWO BLUE-GREEN ALGAE

THE effect of red and far red radiations on various morphogenetic processes is well known in the case of higher plants, ferns, bryophytes and green algae. In blue-green algae light dependent morphogenetic changes in the developmental cycle of *Nostoc* were studied by Lazaroff<sup>1</sup> where red light was shown to be connected with photoinduction and green light, not far red with photoreversal. In the present work we have shown the involvement of red and far red wavelengths during the germination of spores (akinetes) of two blue-green algae.

#### Materials and Methods

*Anabaena* sp. and *Anabaenopsis arnoldii* have been isolated from the brines of Sambhar salt lake of Rajasthan. The culture medium (SS medium) contained dipotassium hydrogen phosphate 0.348 g, potassium nitrate 0.2 g, Sambhar salt crystals 10 g, Sambhar soil extract (4% w/v in distilled water) 10 ml and total volume made up to 1 litre. Iron was added as Fe-EDTA complex<sup>2</sup>, 3 mg/l and trace element solution supplied was that of Fogg<sup>3</sup>, 1 ml/l. The pH of the medium was adjusted to 7.0 for *Anabaena* sp. and 8.5 for *A. arnoldii*, before autoclaving.

Clonal cultures of the algae were raised from single spores plated on agarized SS medium. Algae were generally grown in liquid medium illuminated continuously by 40 w daylight fluorescent lamps, at 28° C ± 2. Whenever spores were required, material from liquid cultures was spread on agar surface and incubated in light. Within 7–10 days or so, all the vegetative cells of the filaments developed into spores. The plates with fully formed

mature spores were scraped and dispersed in a small volume of sterile SS medium and spread on nutrient agar plates and exposed to different colours of light, in boxes fitted with cellophane filters on their lids. The light transmitted by the filters was checked by calibrated hand spectroscope which is as follows: blue—435 nm to 520 nm, green—460 nm to 560 nm, red—600 nm to 660 nm and far red—670 nm to 700 nm. Transfer of petri plates from red to far red and *vice versa* was done in dim blue-green light, 460 nm to 560 nm which was found to be ineffective on the germination process.

### Results

The germination of spores is found to be highest, in white light, 95% in the case of *Anabaena* sp. and 62% in *A. arnoldii* (Table I). Very few spores germinated in complete darkness and also in green and blue lights. In red light, the germination was less than in white light. When the spores previously exposed to red for 24 hr, were transferred to far red, there was an inhibition in germination 90.8% and 78.6% in *Anabaena* sp. and *A. arnoldii* respectively, as compared to the germination in red alone. Further, when spores were initially exposed to far red for 24 hr and then transferred to red, there was a stimulation of germination, 51% in *Anabaena* sp. and 16.5% in *A. arnoldii*. Experiments also done with spore suspensions in liquid medium gave identical results.

TABLE I

Percentage germination of spores of two blue-green algae

| Treatment                    | <i>Anabaena</i> sp. | <i>Anabaenopsis arnoldii</i> |
|------------------------------|---------------------|------------------------------|
| White light (144 h)          | 94.54               | 62.08                        |
| Dark (144 h)                 | 0.89                | 0                            |
| Red (144 h)                  | 48.75               | 34.75                        |
| Far Red (144 h)              | 0.95                | 0.93                         |
| Red (24 h) → Far Red (120 h) | 4.51                | 7.43                         |
| Far Red (24 h) → Red (120 h) | 48.40               | 15.37                        |

### Discussion

Lazaroff<sup>1</sup> studied the effect of different wavelengths of light on the developmental cycle of

*Nostoc muscorum*. He noted that the effects of red radiation (650 nm) can be reversed, not by far red but by radiation of lower wavelengths, broad green region between 480 nm to 560 nm. He showed<sup>2</sup> that the action spectrum for photoactivation matches well with the absorption spectrum of allophycocyanin present in the alga; however, no single pigment could be regarded as receptor for the photoreversal. Our observations on the effect of red and far red can be compared only with the morphogenetic phenomena found in higher plants, ferns, bryophytes and green algae, where the photoinduction and photoreversal are shown to be mediated by phytochrome, a bichromoprotein closely related to blue-green algal pigment phycocyanin. It is not known whether blue-green algae contain phytochrome. In an allied alga *Anabaena cylindrica*, Fay<sup>3</sup> showed that in mature spores, phycocyanin is largely absent, chlorophyll is partly replaced by phaeophytin, the  $\beta$ -carotene content reduced and xanthophyll content increased. A detailed analysis of the pigments present in the spores is very much needed to determine the nature of photoreceptor involved in the germination process.

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### NEW PLANT TYPE IN BLACK GRAM

THE reconstruction of plant type in pulses requires a reduction in their spreading and bushy growth habit not only to raise more plants per unit of land but also to improve the harvest index and reduce the maturity period<sup>2</sup>. In black gram (*Phaseolus mungo* L.) since such a type is not available in natural population, attempts were made to induce such mutations artificially.



Dry seeds of black gram were treated with different doses of X-rays and ethyl methane sulfonate (EMS) singly as well as in combination as described elsewhere<sup>1</sup>. Though a large number of dwarf mutants were found in  $M_2$  generation only one dwarf mutant from 40 kR X-rays and 0.3% EMS treatment was fertile.

The mutant was characterised by a drastic reduction in the length of the internodes, petioles and peduncles (Fig. 1). The leaves were dark green



FIG. 1. (1) Normal, (2) Mutant.

in colour, blades were shrunk into folds and the veins were swollen in addition to the reduced leaf size. The mutant was photoinensitive and mature in 80–85 days like the parent strain. Though there was a drastic reduction in all the vegetative parts, floral organs were not reduced and produced some pods with viable seeds. The mutant bred true in subsequent generations.

To ascertain the mode of inheritance, reciprocal crosses were made between the normal and mutant as described by Sen and Jana<sup>3</sup>. The  $F_1$ 's from all the crosses were normal indicating that the mutant character is recessive. All the  $F_2$  progenies segregated into normal and mutant in 3:1 ratio (Table I) indicating a single gene difference. This

TABLE I  
Inheritance of dwarf mutant in black gram

| Parents               | Segregation in $F_2$ |        | $X^2$<br>(3:1) | $d$     |
|-----------------------|----------------------|--------|----------------|---------|
|                       | Normal               | Mutant |                |         |
| Normal $\times$ Dwarf | 177                  | 53     | 0.47           | 0.5–0.3 |
| Dwarf $\times$ Normal | 150                  | 45     | 0.40           | 0.7–0.5 |

was further confirmed in  $F_3$  as all the dwarf  $F_2$  plants bred true. Out of 10 phenotypically normal  $F_2$  plants, four bred true while the rest segregated like  $F_1$ . The gene symbol Dw/dw was proposed for this allelic pair. The availability of a fertile dwarf type with less vegetative growth offers scope for evolving an ideal plant type described by Jain<sup>2</sup>.

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#### A NEW SPECIES OF *PHYLLACHORA* NITSCHKE APUD FUECKEL ON *ORYZA SATIVA* L. FROM GORAKHPUR (U.P.), INDIA

DURING a survey of plant diseases, plants of water-logged cultivated "Boro" variety of *Oryza sativa* L., in the low lying areas of Gorakhpur were seen infected during the month of October 1973. The infected leaves exhibited black, oval, distinct tar spots (0.5–1.0 mm. dia.), uniformly distributed throughout the lamina (Fig. 1a). The spots first appeared on the upper surface of leaves but were later present on the lower surface also. The plants, which were less submerged, had less of the infection. The sections through the spots of infected host leaves, on examination, revealed them to be perithecia of some species of *Phyllachora*<sup>1</sup>. The perithecia in surface view showed a dot in the centre of each indicating an ostiole but possibly it remains rudimentary even in mature perithecia. The vertically compressed shape of the perithecium, indistinct ostioles, irregular arrangement of ascospores within the ascus and presence of both apical and basal paraphyses are features which are distinct from those reported for the existing species of *Phyllachora*. But for the presence of both apical and basal paraphyses and dark peridium, the structures showed slight resemblance to *Nectria* type centrum as defined by Miller<sup>3</sup> (1949) and Luttrell<sup>2</sup> (1951).

Pathogenicity tests were performed by spraying ascospores cum hyphal aqueous suspension of the fungus on young healthy leaves. Symptoms appeared gradually after 6–8 days.

The specimens of the diseased leaves were examined by Dr. Sivanesan of the C.M.I., Kew, who confirmed the fungus to be a new species of *Phyllachora*, as this fungus does not resemble with the existing species.

The detailed morphological characters of the fungus are as follows :

*Phyllachora gorakhpurensis*, Srivastava and Bhargava, sp. nov.—Perithecia vertically compressed, slightly oval, 100–130  $\mu$  high and

160–230  $\mu$  broad; ostioles not very distinct: situated below upper epidermis as well as lower epidermis (Fig. 1 b). Hymenium basal; asci clavate, bulged in the middle, 45–50  $\mu$  high and 8–16.5  $\mu$  broad; ascospores arranged haphazardly in the ascus (Fig. 1 b and c). Ascospores elliptical, 7–8.5  $\mu$  in diam. (Fig. 1 d). Paraphyses basal as well as pendent from the roof of perithecia (pseudoparaphyses-apical paraphyses). Basal paraphyses projecting beyond the asci, apical reaching upto the base of asci, 1.1–5  $\mu$  broad (Fig. 1 b). Peridium loose, pseudoparenchymatous, dark and distinct except in the basal region (Fig. 1 b).

On living leaves of *Oryza sativa* L. water-logged variety (Gramineae), Bichhia fields, Gorakhpur, October 1973, Leg. Y. N. Srivastava.

*Phyllachora gorakhpurensis*, Srivastava et Bhargava, Spec. nova.—Perithecia perpendiculum compressa; levis ovata, 100–130  $\mu$  alta et 160–230  $\mu$  lata; ores non distincti; situata et sub superam epidermen et super inferam epidermem. Hymenium inferum; asci clavati, extensi in medio, 45–50  $\mu$  alti et 8–16  $\mu$  lati; ascospores ordinati casu inasco (Fig. 1 b et c). Ascospores ellipticales, 7–8.5  $\mu$  in diametere (Fig. 1 d). Paraphyses et inferi et penduli a tectu peritheciae (pseudoparaphyses-paraphyses apicales). Inferiores paraphyses ultra ascum, apicales attingentes lasum asci, 1–1.5  $\mu$  lati (Fig. 1 b). Peridium mobile pseudoparenchymatous, niger et distinctus praeter in inferiore parte (Fig. 1 b).

wealth Mycological Institute, Kew, England, for their help during the identification of the fungus. They are also thankful to Rev. Fr. Lawrence Mendonca, Parish Priest, Catholic Church, Gorakhpur, for Latin diagnosis and to Dr. Y. B. Singh, Principal, and Dr. G. C. Srivastava, Head, Botany Department, St. Andrew's College, for providing laboratory facilities to senior author. Thanks are also due to Dr. A. B. Sinha and Mr. J. S. Srivastava, for their help and to U.G.C. for financial assistance to one of us (Y. N. S.).

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#### STIGMATIC EXUDATES AND PLANT STERILITY: A CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC STUDY

THE use of ultraviolet spectrophotometry in the analysis of stigmatic exudates has been reported by Martin<sup>1,2</sup> and others<sup>3,4,5</sup>. The authors also observed<sup>3,4</sup>, in the UV profiles of the stigmatic exudates of a sterile mutant of *Impatiens sultani*, the absence of a peak characteristic of fertile plants. A summary of the data, obtained from a spectrophotometric and chromatographic analysis of a variety of mutants of *Impatiens*, presented here suggest a positive correlation between stigmatic exudates defective of some compounds absorbing UV light in the 250–300 nm region and plant sterility.

Association of specific UV peaks and fertility was tested in a dozen varieties of *Impatiens* available in our Botanical Gardens, but four colour mutants of *I. sultani* (known as 'Pink', 'Crimson', 'Orange' and 'Magenta') and one of *I. beddomei* (called 'White') showing various degrees of sterility were chosen for a detailed study. *In vitro* germination tests indicate that the approximate pollen viability of mutants vary from 2% in Pink to almost 100% in Magenta. Fruit setting is observed in Magenta, Crimson and Orange while Pink and White are sterile. *In vivo* observations revealed that viable pollen germinate on the stigmas of Magenta, Crimson and Orange but not on those of White and

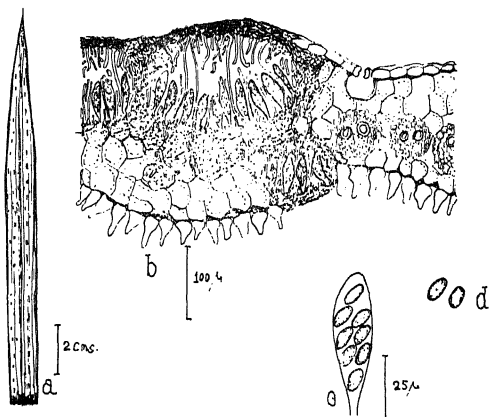


FIG. 1. a, Infected *Oryza sativa* leaf showing tar spots. b, V.T.S. of the host leaf through tar spots showing perithecia. c, Single ascus. d, Ascospores.

In viventibus foliis *Oryzae sativae* L. aquae collectae (Gramineae), in agris Bichhiae, Gorakhpur, mense Octoberi 1973, Leg. Y. N. Srivastava.

The type specimen has been deposited in the Herb. I.M.I., Kew, at No. 187024.

The authors are thankful to Dr. A. Johnston, Director and Dr. Sivanesan, Mycologist, Common-

Pink. For convenience, the former are referred to here as fertile and the latter as sterile.

UV absorption spectra of stigmatic exudates was studied by immersing 25 stigmas of each mutant into 4 ml of ethanol for 10 sec. Stigmatic surfaces do not show any sign of damage as a result of this treatment. The extracts showed constant and repeatable profiles when analyzed in a Beckman DU<sub>2</sub> Spectrophotometer. All mutants showed high absorbance and discernible peaks in the region below 250 nm but this had been reported for all genera and species examined by Martin<sup>1</sup> and

paper chromatography, *n*-butanol-acetic acid-water (4 : 1 : 5) mixture was found to be most suitable.  $R_f$  values of spots were taken as the average of six replicated extraction and chromatography series. The mutants showed variations in the number, the size and the distribution of spots. Various location reagents showed strong presence of anthocyanins in Crimson, while traces of free sugars were detected in Orange. However, the outstanding difference noted in the chromatograms of various mutants was the absence of spots 2 and 3 in sterile mutants (Table I).

TABLE I

*UV absorption peaks and  $R_f$  values of stigmatic extracts of Impatiens mutants\**

|                | Orange (Fertile) |                      |              | Pink (Sterile)  |                      |              |
|----------------|------------------|----------------------|--------------|-----------------|----------------------|--------------|
|                | $R_f$<br>values  | Absorption peak (nm) |              | $R_f$<br>values | Absorption peak (nm) |              |
|                |                  | Without<br>NaOH      | With<br>NaOH |                 | Without<br>NaOH      | With<br>NaOH |
| Crude extracts | ..               | 261                  | 270          | ..              | ..                   | ..           |
| Spot 1         | 0.29             | 279                  | 280          | 0.29            | 279                  | 279          |
| Spot 2         | 0.49             | 246                  | No peak      | ..              | ..                   | ..           |
|                |                  | 272                  | No peak      | ..              | ..                   | ..           |
|                |                  | 300                  | 290          | ..              | ..                   | ..           |
| Spot 3         | 0.60             | 278                  | 282          | ..              | ..                   | ..           |
| Spot 4         | 0.86             | 277                  | 285          | 0.86            | 277                  | 284          |

\* Data from a representative of fertile (Orange) and sterile (Pink) mutants only are given in this table. Other mutants belonging to both categories have similar values.

others<sup>4,5,6</sup> and therefore has no relevance in comparisons. All fertile mutants, living either in natural habitats or in green houses, yielded similar results with respect to the principal peak around 260 nm with a red shift of less than 10 nm in alkaline extracts. By contrast, the sterile mutants—Pink and White—do not reveal any peak in the 250–300 nm region indicating the absence of compounds that absorb light in this range. Absence of these compounds from the stigmatic exudates is associated with their failure to support stigmatic pollen germination and consequent failure to set seeds.

For chromatography, ethanolic extracts from 500 stigmas of each mutant were used. Of the different solvent systems employed in descending

Spots 1–4 were cut out from the chromatograms, eluted in ethanol and spectrophotometrically analyzed. Eluates of all spots show specific peaks. Screening the alkaline extracts shows no shift or no peaks in some cases while others record positive or negative shifts. As summarized in Table I, spectral data suggest the absence of four species of compounds in the stigmatic exudates of sterile mutants. Responses of spots to various colour tests, cited by Harborne<sup>7</sup> and the small bathochromic shifts of the eluates imply that some of these compounds are simple phenolics but further characterization was not possible.

The point of interest here is the total absence, or presence only in low concentrations, of compounds

of spots 2 and 3 in sterile mutants. This absence is associated with lack of pollen germination and seed setting. It has already been reported<sup>6</sup> that the sterility of Pink is not due to aberrations in megagametogenesis but due to failure of fertilization. For this paper, we followed the embryological events of other mutants and found that the sequences, in all of them are parallel until the time of pollination, after which the ovules of sterile mutants gradually aborted. In addition, the present study showed that intraovarian pollination failed to develop seeds both in fertile and sterile mutants. Perhaps this emphasizes the importance of stigmatic surfaces in pollen germination. In recent years, stigmatic surfaces<sup>8</sup>, pollen emissions<sup>9</sup>, and pollen wall degradation<sup>10</sup> have received attention from the angle of pollen-pistil interactions. Studies on female sterility and incompatibility do not usually take into consideration the role of defective stigmatic fluids; we suggest that this aspect is worthy of further investigation.

We thank Prof. C. A. Ninan, Head of the Department of Botany, for facilities and Dr. D. J. Francis, Department of Chemistry, Kerala University, for critically reading the manuscript. C.P.T. is grateful to CSIR for a fellowship.

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#### A NEW SPECIES OF *PHYLLOSTICTA* CAUSING LEAF SPOT OF *NYCTANTHES ARBOR-TRISTIS*

THIS leaf spot disease was earlier described by the authors<sup>1</sup>. The pathogen, *Phyllosticta* sp., has been compared with other known species and it has been found to be a new taxa. It attacks the leaves of *Nyctanthes arbor-tristis* L. Its morphological characters have been recorded on *Asthana* and *Hawkers medium* 'A'. It has been named *Phyllosticta azadii* sp. n. after Shri Chandrashekher Azad.

#### *Phyllosticta azadii* sp. n.

Pycnidia were observed on older spots. Hyphae septate, buffy brown, branched, 2.84–4.26  $\mu$  in thickness; pycnidia olive-brown, ostiolate, mostly globose, 92.3–143.42  $\mu$  in diameter (Fig. 1 A and B); conidiophores short, hyaline; conidia hyaline, one-celled, oval to cylindrical, 2.84–7.10  $\times$  1.42–2.84  $\mu$  in size (Fig. 1 C).

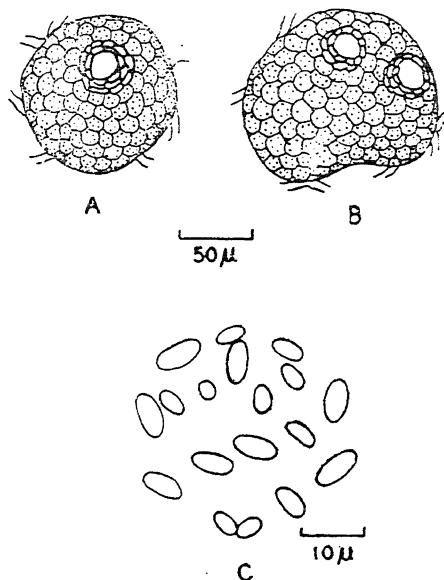


FIG. 1. *Phyllosticta azadii*—A, a single pycnidium; B, two pycnidia fused and C, conidia.

The type species was collected from Chandrashekher Azad Park, Allahabad, India. It infected the leaves of *Nyctanthes arbor-tristis* L. The type culture has been deposited in CMI, Kew, England, No. IMI, 137489.

Inoculations of this species were successful on the leaves of other garden and wild plants growing in the vicinity. Most of them, *Alocasia* sp., *Bougainvillea spectabilis*, *Jasminum arborescens* and *Jasminum sambac* as well as *Achlypha indica* and *Boerhaavia diffusa* were susceptible to this infection.

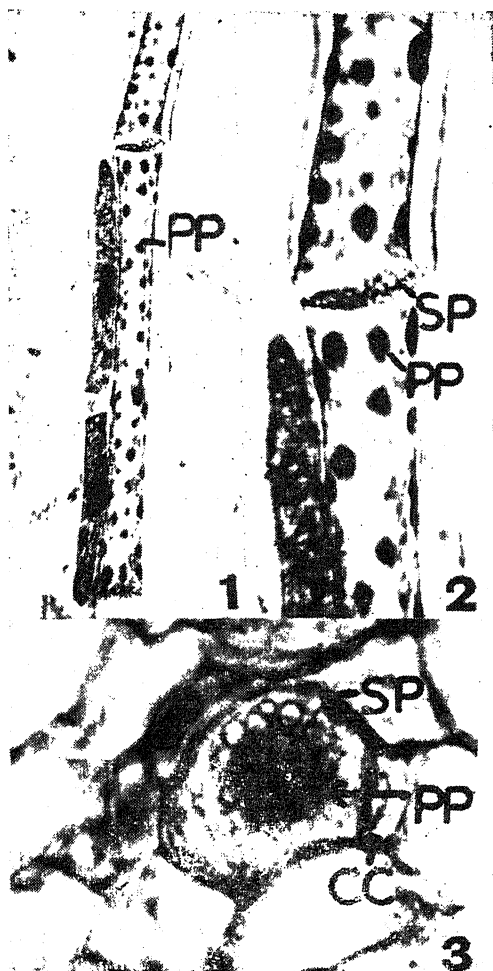
Thanks are due to Dr. C. Booth, Director and Dr. Sutton, Mycologist, CMI, Kew, England, for confirming the identity of the fungus, to C.S.I.R. for financial assistance and to the Head of the Botany Department for laboratory facilities.

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Allahabad, India, March 14, 1975 R. N. TANDON.

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# UNDISPERSED P-PROTEIN BODIES IN THE MATURE SIEVE TUBE ELEMENTS OF THE PETIOLE OF *LAGENARIA*

PETIOLE of *Lagenaria leucantha* (Duch.) Rusby was fixed in FAA<sup>1</sup>. Sections and macerated material were stained with a combination of tannic acid, ferric chloride, and resorcin blue<sup>2</sup>. Mercuric bromphenol blue<sup>3</sup> was used to confirm the proteinaceous nature of the P-protein.



FIGS. 1-3. Fig. 1. Enucleate sieve tube element with P-protein bodies,  $\times 155$ . Fig. 2. A magnified view showing sieve plate with pores and P-protein bodies appressed to the peripheral cytoplasm,  $\times 665$ . Fig. 3. Transection showing a sieve plate with callose lined pores and a P-protein body,  $\times 1490$ .

(SP, Sieve plate; CC, Companion cell; PP, P-protein body.).

The petiole of *Lagenaria leucantha* shows about 9-10 vascular bundles with outer and inner phloem. Some of the mature sieve tube elements of the external phloem showed numerous spherical or oval P-protein bodies. They are normally peripherally situated and seen appressed to the peripheral cytoplasm (Figs. 1, 2). Test with bromphenol blue indicated their proteinaceous nature. Sieve tube elements with such undispersed P-protein bodies were enucleate and randomly scattered in the external phloem (Fig. 1). A transverse sieve plate shows numerous callose lined sieve pores (Fig. 3). Number of pores varies from 42-65 and the diameter of the pores, 3-5  $\mu\text{m}$ . Most of the undispersed P-protein bodies are not disturbed from their peripheral position in the sectioned and macerated material. Occasionally they may lie against the sieve plate (Fig. 3), but they do not constitute a P-protein plug which is observed in a sieve tube element where P-protein was in dispersed state.

Similar undispersed P-protein bodies in the mature sieve tube elements were reported by Cronshaw and Esau<sup>4</sup> in *Cucurbita*. But they were extra fascicular sieve tube elements in petiole and stem of *Cucurbita*. In the petiole of *Lagenaria leucantha* similar sieve tube elements form a part of the external phloem of the vascular bundle. We have failed to observe them in the internal phloem. Dispersed P-protein is a normal structural feature in a mature functioning sieve tube element. The presence of P-protein body is usually a feature of developing sieve tube element. Its presence in the mature sieve tube elements of the petiole of *Lagenaria* not only reflects varying behaviour of P-protein, but also complicates the determination of its role in translocation.

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## REVIEWS AND NOTICES OF BOOKS

**Cell Biology.** By E. J. Ambrose and Dorothy, M. Easty. (The English Language Book Society and Nelson, London). 1973. Pp. 500. Price £ 1.35.

The field of cell biology encompasses many disciplines, ranging from genetics to biochemistry to biophysics. How much of this vast amount of information will be useful to a beginner in the field? The author's task has been one of selection and compression to create an introductory textbook of cell biology which is sufficiently informative, but not cumbersome.

Chapter 1 in Part 1 is intended to provide a general introduction to students who have had no previous experience of biology. On the other hand, chapter 2 is intended as an introduction to those who have studied biology but will have little previous knowledge of the rather specialized type of chemistry which is needed to follow the recent developments in molecular biology and biochemistry. Both chapters include a summary of the practical methods used in cell biology, molecular biology and biochemistry which the student will encounter in practical courses.

Part 2 describes the structure and function of various cellular components which are found almost universally in micro-organisms, and in plant and in animal cells. The function of the cellular components in interphase cells is specially dealt with, since it is in the interphase nucleus that the genes of the chromosomes are actively engaged in the control of cellular synthesis.

Part 3 deals with the integrated function of growing cells, in the cell cycle, in mitosis, in the behaviour of germ line cells and the genetics of bacteria, and plant and animal cells.

In Part 4 attention turns to the dynamic function of whole cells, cytoplasmic and whole cell movements, cellular interactions, and differentiation in development. Developmental biology provides a link between cell biology and general biology, and an attempt has been made to show the relevance of a cellular approach in relation to courses in general biology. In Part 5 the authors consider the formation of biological structures from biochemical building units, and discuss the origin of the latter.

A few random comments are offered: the references at the end of each chapter appear to be both relevant and current. The illustrations and tables are of good quality and complement the text. In conclusion I would like to state that I enjoyed

reading this book and believe that it is well designed to fit the needs of students entering the field of cell biology; however, it is not intended as a reference book. In addition, the book should be of value to biologists and chemists who desire a concise, readable text of cell biology which includes many advances in molecular biology.

T. RAMAKRISHNAN.

**Annual Review of Biochemistry** (Vol. 43). Edited by E. E. Snell, P. D. Boyer, A. Meister and C. C. Richardson. (Annual Reviews, Inc., Palo Alto, California, U.S.A.), 1974. Pp. viii + 1085. Price : USA \$ 16.00 ; Foreign : \$ 16.50.

The major thrust of Life Sciences research is now towards interpreting biological phenomena in molecular terms; a distinct shift from prokaryote to eukaryotic systems is also being observed. Accordingly, studies on nucleic acid replication, translational and transcriptional controls and membrane structure have gained momentum.

The 1974 *Annual Reviews in Biochemistry* highlights these trends in modern biochemical research. The articles on eukaryotic messenger RNA (Brawerman) and the methods of gene isolation (Brown and Stern) stem from the findings of the presence of poly-A in messenger RNA and the development of a variety of techniques, including gene enrichment methods, for isolation of gene segments. Considerable amount of information on DNA replication in eukaryotic systems has been possible because of the studies with circular DNA (Kasamatsu and Vinograd). The article on animal RNA viruses; genome structure and function (Shatkin), illustrates the diversity found among animal RNA viruses providing a catalog of model systems for investigators, interested in eukaryotic cells. The evaluation of the different methods for DNA sequencing (Salser) and the article on selectivity of gene transcription (Chamberlin) clearly emphasise the influence of gene structure on the primary process of transcription. An important method of translational control is achieved by protein turnover, highlighted in the article on intracellular protein degradation in mammalian and bacterial cells (Goldberg and Dice). The articles on biochemistry of bacterial cell envelopes (Braun and Hantke), bacterial transport (Boos), Synaptic macromolecules: Identification and metabolism (Barondes), membrane receptors (Cuatrecasas), the sodium-potassium ATPase (Dahl and Hokin), the molecular organization of

membranes (Singer), the biosynthesis of mitochondrial proteins (Schatz and Mason) summarise in good detail the progress in membrane interaction and biochemistry. The reviews entitled metabolic transformation of fatty acids (Fulco), phosphoglyceride metabolism (Vanden Bosch), regulation of amino acid decarboxylation (Morris and Fillingame), peptide hormones (Tager and Steiner), biosynthesis of water-soluble vitamins (Plaut *et al.*), regulation of steroid biosynthesis (Dempsey), are highly informative and cover the ground of classical biochemistry.

It is heartening to read the reviews on the biochemistry of drug dependence (Takemori) and biochemistry of mammalian fertilization (McRorie and Williams), which emphasize that these problems of pharmacology and physiology can now be better understood in molecular terms. The other important reviews cover a range of subjects such as peptide synthesis, application of X-ray methods and electron microscopy to the study of macromolecular structure and interaction, collagen biosynthesis, mechanism of enzyme action, unusual polysaccharides and fungal sex-hormones.

The *Annual Reviews of Biochemistry* is always awaited eagerly by all students of Life Sciences. The 1974 Volume amply justifies this sentiment in terms of the quality and quantity of the scientific information.

G. PADMANABAN.  
H. R. CAMA.

**Human Physiology: The Mechanisms of Body Function** (II Edition). By Arthur J. Vander, James, H. Sherman and Dorothy S. Luciano. (Tata McGraw-Hill Publishing Company, Ltd., New Delhi). 1975. Pp. x + 614, Price Rs. 39-00.

The overall organization and approach of the book is based upon a group of themes:

All phenomena of life, no matter how complex, are ultimately describable in terms of physical and chemical laws; certain fundamental features of all function are shared by all cells and, in addition, constitute the foundation upon which the specialization is built and the body's various coordinated functions like circulation, respiration, etc., result from the precise control and integration of specialized cellular activities.

These viewpoints are established with suitable illustrations in the introductory chapter. The book then progresses from the cell to the total body, utilizing at each level of increasing complexity, the information and principles developed previously.

Part I is devoted to an analysis of basic cellular physiology and the essential physics and chemistry

required for its understanding and deals with chemical composition of the body, movements of molecules across cell membranes, energy and cellular metabolism, protein synthesis, heredity and cell development.

Part II analyses the concept of the body's internal environment, the nature of biological control systems and the properties of the major specialised cell types—nerve, muscle and gland.

Part III, then, analyses the coordinated body functions, circulation, respiration, regulation of water and electrolyte balance, digestion, energy balance and reproduction.

Defence mechanisms of the body, processing of sensory information, control of body movement and consciousness and behaviour are other highly informative chapters.

The book is intended for undergraduate students regardless of their scientific background. All topics are featured in an extremely interesting manner. The claim of the authors that besides providing an up-to-date information on the mechanisms of body function, the approach has been to make the student think rather than simply memorize, is amply justified by the presentation of the concepts and explanations, along with the considerable gaps in our current understanding of Human Physiology.

M. SIRSI.

## ANNOUNCEMENT

### Symposium on Infrared Materials and Devices

A Symposium on Infrared Materials and Devices will be held at Solid State Physics Laboratory, Delhi 110 007, on March 11 and 12, 1976. The main emphasis in the Symposium will be on (a) IR materials; (b) IR devices. The deadline, for receiving the abstract is 15th October 1975 and for receiving manuscript 1st December 1975.

Further details can be had from the Chairman of the Symposium.

## ERRATA

Table II of the note entitled "An Unreported Linkage Group in Rice (*Oryza sativa* L.)" appeared in *Current Science*, May 20, 1975, Vol. 44, No. 10, Page 356 under column WP, for 799 read 99 and for 108-94 read 185-94.

In the note entitled "Cytogenetics of Semi-arid Plants" by A. K. Singh, appeared in *Current Science*, July 20, 1975, Vol. 44, No. 14, Page 511, for "Cytological Studies in *Corallocarpus conocarpus* Dalz & Gibs." read "*Corallocarpus epigaens* Rottle & Willd. of Cucurbitaceae".

# Current Science

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## PUBLICATIONS OF FERTILIZER CORPORATION OF INDIA AVAILABLE FOR SALE

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# KINETICS AND MECHANISM OF THE NEUTRAL HYDROLYSIS OF 2, 4-DICHLOROPHENYL DIHYDROGEN PHOSPHATE

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## ABSTRACT

Hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate has been studied in aqueous medium (*ca.* pH 7.0), at 98°. During the hydrolysis, the pH of the solution varies from pH 7.0 to pH 3.0. Agreement of the rate of neutral hydrolysis, with specific mononegative rate, shows that the reaction proceeds *via* mononegative species. The reaction proceeds with bimolecular nucleophilic attack of water on phosphorus of the reactive mononegative species involving phosphorus-oxygen bond fission. The effect of temperature, solvent and reagents on the reaction rate have been studied to give extra support to probable reaction mechanism.

**T**HE well-acknowledged role and wide applicability of reactions of organic phosphates in various branches of chemistry, has inspired tremendous attention towards their reaction kinetics of hydrolysis during the last two decades<sup>1</sup>. Almost no kinetic data of the neutral hydrolysis of phosphates are available. Thus investigation of the neutral hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate, which is a synthetic plant hormone<sup>2</sup>, is undertaken with a view to finding the influence of the substitution of hydrogen atoms by chlorine atoms in ortho and para positions to the phosphate side chain of the ester on the reaction mechanism.

## MATERIALS AND METHODS

2, 4-dichlorophenyl dihydrogen phosphate was prepared from the 2, 4-dichlorophenol and phosphorus oxychloride by the method devised by Maguire and Shaw<sup>3</sup>. The product, 2, 4-dichlorophenyl phosphorodichloridate, b.p. 115°/1.5 mm, was slowly added to warm water with stirring; when cooled the solution deposited, 2, 4-dichlorophenyl

## RESULTS AND DISCUSSION

The neutral hydrolysis (*ca.* pH 7.0) has been studied at 98° and the rate constant has been found to be  $13.7 \times 10^{-3} \text{ min.}^{-1}$ . Identification of the reactive species has been done from the nature of the pH-log rate profile and pK values for various equilibria<sup>5</sup>. The hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate has also been studied in buffers in the range pH 1.24 to 7.46 at 98°. The pH-log rate profile shows the maximum rate ( $14.74 \times 10^{-3} \text{ min.}^{-1}$ ) in buffer of pH 4.5 and minimum rate ( $7.36 \times 10^{-3} \text{ min.}^{-1}$ ) at pH 0.2 and these have been explained on the basis of reactive mononegative and neutral species respectively<sup>1,6</sup>. The dinegative species in the buffer of pH 7.46 are found to be inert ( $0.2 \times 10^{-3} \text{ min.}^{-1}$ )<sup>6</sup>. The 2, 4-dichlorophenyl dihydrogen phosphate seems to be dissociated in two steps and the respective pK<sub>1</sub> (1.5) and pK<sub>2</sub> (7.5) have been calculated from the minimum rate at pH 0.2 and maximum rate at pH 4.5 respectively<sup>6</sup>.

Neutral species  $\xrightarrow{\text{pH } 0.2 \text{ to } 4.5}$  Mononegative species + H<sup>+</sup>  $\xrightarrow{\text{pH } 4.5 \text{ to } 7.46}$  Dinegative species + H<sup>+</sup>

dihydrogen phosphate which separated from toluene, m.p. 65° (Found C, 28.90; H, 2.50; P, 13.0. Required C, 29.65; H, 2.05; P, 12.74%).

## PROCEDURE

Kinetic runs were carried out at  $98^\circ \pm 0.05^\circ$  employing  $5.0 \times 10^{-4} \text{ M}$  solution of 2, 4-dichlorophenyl dihydrogen phosphate in aqueous medium (*ca.* pH 7.0). The estimation of reaction product (Inorganic phosphate) was carried out by Allen's

Comparative data of the hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate in neutral medium, *ca.* pH 7.0 ( $13.7 \times 10^{-3} \text{ min.}^{-1}$ ) and in buffer of pH 7.46 ( $0.2 \times 10^{-3} \text{ min.}^{-1}$ ) show that the rate of the neutral (pH 6.6) hydrolysis is 70 times more than the hydrolysis in buffer of 7.46. Such a big rise in rate seems to be due to fast shifting of equilibrium in the range pH 4.5 to 7.46, in the reverse direction.

Mononegative species  $\xrightarrow{\text{Fast in buffers}}$  Dinegative species + H<sup>+</sup>  
 $\xrightarrow{\text{Fast in aqueous medium}}$

modified method<sup>3</sup> using "Systronix" Photoelectric Colorimeter. All chemicals used were of A.R. quality. Dioxan was purified<sup>4</sup>.

Probably conversion of existing dinegative species (which are inert in buffers) to mononegative, by protonation is kinetically fast in aqueous medium. It has been confirmed by measuring the pH of the solution during hydrolysis, which decreases from 6.6 to 3.0.

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Arrhenius parameters (Table I) for the neutral hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate were determined by carrying out kinetic runs at 80°, 90° and 98° in water. These results are consistent with bimolecular<sup>7</sup> nature of the reaction. Agreement of these Arrhenius parameters with those calculated for mononegative species by carrying out kinetic runs at pH 4.17 (Table I) supports that the hydrolysis proceeds *via* mononegative species.

TABLE I

Arrhenius parameters for the hydrolysis of  
2, 4-dichlorophenyl dihydrogen phosphate

| Parameters          | Medium                             |                                    |
|---------------------|------------------------------------|------------------------------------|
|                     | pH 4.17                            | Aqueous (ca. pH 7.0)               |
| E                   | 20.9 K.cal/mole                    | 22.0 K.cal/mole                    |
| A                   | $4.8 \times 10^8 \text{ sec}^{-1}$ | $2.3 \times 10^9 \text{ sec}^{-1}$ |
| $\Delta_s^\ddagger$ | -21.2 e.u.                         | -18.2 e.u.                         |

Study of the reaction in solvent mixtures has been carried out at 80° by Grunwald and Winstein<sup>8,9</sup> method for supporting molecularity of the reaction. Data are summarised in Table II. Sensitivity constant (*m*) was found to be 0.10. Such a small magnitude of slope (much less than unity) is indicative of bimolecular<sup>8,9</sup> nucleophilic attack of water on reactive species.

TABLE II

Solvent effect of neutral hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate in dioxan-water, *v/v* at 80°

| Percentage of dioxan ( <i>v/v</i> ) | Dielectric constant at 25° | Ionising power Y | $k \times 10^3 \text{ min}^{-1}$ | $k/k_0$ |
|-------------------------------------|----------------------------|------------------|----------------------------------|---------|
| 60                                  | 27.00                      | 0.715            | 2.30                             | 3.28    |
| 50                                  | 35.60                      | 1.361            | 2.49                             | 3.55    |
| 40                                  | 44.30                      | 1.945            | 2.71                             | 3.87    |
| 20                                  | 62.20                      | 2.880            | 3.00                             | 4.28    |
| 00                                  | 78.50                      | 3.493            | 2.70                             | 3.85    |
| 80% Ethanol                         | ..                         | ..               | 0.70                             | 1.00    |

Kinetic runs were carried out in aqueous medium (ca. pH = 7.0) for the further support to molecularity of the reaction of 2, 4-dichlorophenyl dihydrogen phosphate in presence of reagents of graded nucleophilic power (Table III). The change in rates

TABLE III

Hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate in aqueous medium at 98° in presence of nucleophiles

| Nucleophilic reagent             | Nucleophilicity <sup>11</sup> constant <i>n</i> | Concentration of the reagent | $k_e \times 10^3 \text{ min}^{-1}$ | $k_e/k_0$ |
|----------------------------------|---|------------------------------|------------------------------------|-----------|
| CH <sub>3</sub> COO <sup>-</sup> | 2.72  | 0.1                          | 1.63                               | 0.12      |
| CH <sub>3</sub> COO <sup>-</sup> | 2.72  | 0.5                          | 0.43                               | 0.03      |
| CH <sub>3</sub> COO <sup>-</sup> | 2.72  | 1.00                         | 0.27                               | 0.02      |
| H <sub>2</sub> O                 | 0.00  | ..                           | 13.71                              | 1.00      |
| Cl <sup>-</sup>                  | 3.04  | 0.10                         | 15.36                              | 1.12      |
| Br <sup>-</sup>                  | 3.89  | 0.10                         | 16.21                              | 1.18      |
| I <sup>-</sup>                   | 5.04  | 0.10                         | 18.26                              | 1.33      |

with the change in reagents of graded nucleophilic power (Fig. 1) accounts for the bimolecular attack

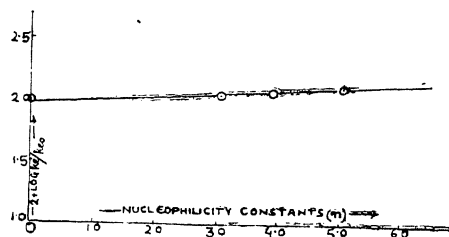


FIG. 1. Hydrolysis of 2, 4-dichlorophenyl dihydrogen phosphate in aqueous medium at 98° in presence of nucleophiles (Swain-Scott relationship). Rate constant for hydrolysis of the compound in given anion;  $k_0$ , Rate constant for the hydrolysis of the same compound in water; *n*, Nucleophilicity constant.

of the reagent on the substrate<sup>12,13,14</sup> as has been observed by Swain and Scott<sup>14</sup>. The rate constants in the presence of the acetate ion (Table III) have been found to be an exception because of its dominating basicity<sup>15</sup>. The decrease in rate constants with the

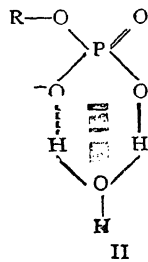
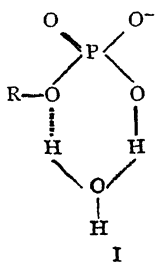
increase in ionic concentration of the acetate ions, as shown in Table III, is expected to be due to decrease in the concentration of the mononegative species in the pH range 4.5 to 7.46<sup>6,16-17</sup>.

Kinetic runs have also been carried out in presence of cations of varying ionic radii as shown in Table IV. Increase in rates with the increase in ionic radii has been attributed to be due to specific salt effects<sup>6</sup>. Probably, electrostatic attraction of cation with -ve oxygen of reactive mononegative species reduces the -ve character of the reactive species. This effect also decreases with the decrease of the charge density, i.e., with increase of ionic radii<sup>18-20</sup>.

TABLE IV  
Hydrolysis of 2,4-dichlorophenyl dihydrogen phosphate in aqueous medium at 98° in presence of cations

| Cations         | Ionic <sup>18</sup><br>radii<br>Å | Concentration<br>of reagent<br>(M) | $k_e \times 10^3$<br>min. <sup>-1</sup> |
|-----------------|-----------------------------------|------------------------------------|---|
| H <sup>+</sup>  | 0.28                              | 0.1 HCl                            | 7.57                                    |
| Li <sup>+</sup> | 0.60                              | 0.1 LiCl                           | 13.24                                   |
| Na <sup>+</sup> | 0.95                              | 0.1 NaCl                           | 15.36                                   |
| K <sup>+</sup>  | 1.33                              | 0.1 KCl                            | 20.46                                   |

As regards bond fission during neutral hydrolysis, phosphorus-oxygen rather than carbon-oxygen is more probable, because resonance stabilised phenoxide ion<sup>21</sup> is formed, while carbon-oxygen bond fission would have given rise to unstable cation intermediate. Electron attracting groups in the aryl part should facilitate phosphorus-oxygen bond fission<sup>21</sup>, whereas, carbon-oxygen bond fission should be inhibited.



(R=2-Cl, 4-Cl, C<sub>6</sub>H<sub>5</sub>-)

The reaction paths of neutral hydrolysis via mononegative species may also be represented by

rapid formation of hydrogen bonded complexes (I) and (II) with water, which readily decompose with phosphorus-oxygen bond fission, (II) is preferred over (I) since the rate of hydrolysis increases with electron attracting power of the substituent<sup>22</sup>, which will not favour hydrogen bonding as shown in I.

The probable reaction paths of the neutral hydrolysis, consistent with the experimental data, may be formulated as in Chart I shown below:

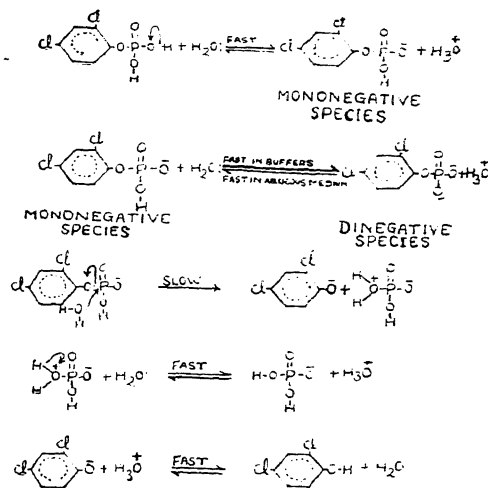


CHART 1. Mechanism of the neutral hydrolysis of 2,4-dichlorophenyl dihydrogen phosphate.

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## DIELECTRIC CONSTANTS AND EFFECTIVE IONIC CHARGES OF SOME CUBIC NITRATES

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## ABSTRACT

Low frequency dielectric constant measurements have been made on die-pressed powder samples of  $\text{Sr}(\text{NO}_3)_2$ ,  $\text{Ba}(\text{NO}_3)_2$  and  $\text{Pb}(\text{NO}_3)_2$ . The dielectric constants (corrected to crystal density) are 5.17, 4.59 and 11.90 respectively. These dielectric constants yield the values 0.84, 0.71 and 1.04 respectively for the Szigeti charges.

THE nitrates of Sr, Ba and Pb form an isomorphous group of crystals with cubic structure. Krishnan<sup>1</sup> has reviewed the data regarding several physical properties of these crystals. However there is meagre information about their dielectric constants. The few values that are available in literature show considerable scatter. Thus we have the values 5.33<sup>2</sup> and 5.83<sup>3</sup> for  $\text{Sr}(\text{NO}_3)_2$ , 4.95<sup>2</sup> and 5.9<sup>4</sup> for  $\text{Ba}(\text{NO}_3)_2$  and 16.8<sup>2</sup>, 15.9<sup>3</sup> and 37.7<sup>4</sup> for  $\text{Pb}(\text{NO}_3)_2$ . In view of these large differences between the values from different sources, a systematic redetermination of the dielectric constants of these materials has been carried out.

Pure chemicals obtained from B.D.H. were used for preparing die-pressed samples and measurements were made at various frequencies following the experimental technique described earlier<sup>5</sup>. Typical curves between the dielectric constant ( $\epsilon_p$ ) of the powder sample and the frequency ( $f$ ) at which the measurement was made are given in Fig. 1. The implications of such curves have been discussed earlier<sup>5</sup> as also, the procedure for obtaining the dielectric constant ( $\epsilon$ ) of the solid from the dielectric constant ( $\epsilon_p$ ) of a powder sample with packing fraction  $\delta$ . The values of  $\epsilon$ , thus obtained, are given in Table I.

The bonding in these crystals is of some interest. These crystals are essentially ionic. From electronegativity considerations the bonding in  $\text{Ba}(\text{NO}_3)_2$  should be more ionic than that in  $\text{Sr}(\text{NO}_3)_2$ . From I.R. spectroscopic studies, Brooker *et al.*<sup>6</sup> conclude that the bonding in these crystals is highly ionic.

TABLE I

*Dielectric constants, input physical properties for eq. (1) and effective ionic charges of  $\text{Sr}(\text{NO}_3)_2$ ,  $\text{Ba}(\text{NO}_3)_2$  and  $\text{Pb}(\text{NO}_3)_2$*

|                            | $r$<br>(Å) | $v$<br>(Å <sup>3</sup> ) | $\beta$<br>(10 <sup>-12</sup><br>cm <sup>2</sup><br>dyne <sup>-1</sup> ) | $n^2$ | $\epsilon$ | $q^*$ |
|----------------------------|------------|--------------------------|--|-------|------------|-------|
| $\text{Sr}(\text{NO}_3)_2$ | 3.54       | 117.7                    | 2.95   | 2.53  | 5.17       | 0.84  |
| $\text{Ba}(\text{NO}_3)_2$ | 3.73       | 133.3                    | 4.26   | 2.47  | 4.59       | 0.71  |
| $\text{Pb}(\text{NO}_3)_2$ | 3.62       | 121.1                    | 3.21   | 3.17  | 11.90      | 1.04  |

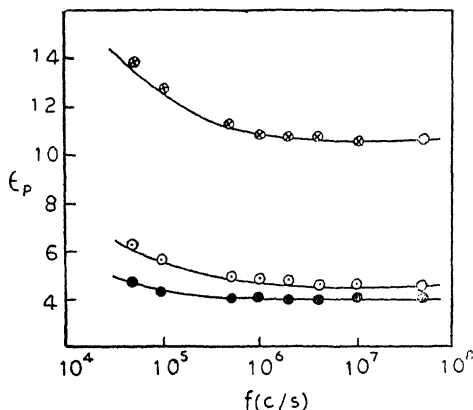


FIG. 1. Plot between the frequency ( $f$ ) and the dielectric constant ( $\epsilon_p$ ) of the powder samples (with  $\delta = 0.9$ ) of  $\text{Sr}(\text{NO}_3)_2$   $\odot$ ,  $\text{Ba}(\text{NO}_3)_2$   $\bullet$  and  $\text{Pb}(\text{NO}_3)_2$   $\otimes$ .

However, from the intensities of the Raman lattice lines, it has been concluded<sup>1,7</sup> that  $\text{Ba}(\text{NO}_3)_2$  is less ionic than  $\text{Sr}(\text{NO}_3)_2$ . Again, the inter-atomic distances in the structures do not reveal any significant difference between  $\text{Sr}(\text{NO}_3)_2$  and  $\text{Ba}(\text{NO}_3)_2$  to suggest stronger binding in the latter. However, on the basis of the melting points, solubilities, Gruneisen constants, elastic constants, photo-elastic constants and magnetic anomaly factors, Krishnan<sup>1</sup> concludes that  $\text{Ba}(\text{NO}_3)_2$  is less ionic than  $\text{Sr}(\text{NO}_3)_2$ .

Useful information about the bonding in crystals can be obtained from the dielectric constant which yields values of the effective ionic charge. We have used the dielectric constants obtained in the present work to evaluate the effective ionic charge of these nitrates. By combining the two well-known Szigeti relations, we obtain<sup>8,9,10</sup> the equation :

$$q^* = [3v/ze] \{ 3(\epsilon - n^2)/4\pi\beta(\epsilon + 2)(n^2 + 2) \}^{1/2} \quad (1)$$

Here  $q^*$  is the effective charge per electron,  $v$  the volume per ion pair,  $z$  the formal valence,  $e$  the electronic charge,  $r$  the inter-ionic distance,  $\epsilon$  the dielectric constant,  $n$  the refractive index and  $\beta$  the compressibility. There are two metal-to-nitrate-ion distances in this structure. As such a weighted average of these distances has been used for  $r$ . The value of  $v$  and  $r$  are obtained from Wyckoff<sup>11</sup>. The value of  $\beta$  are calculated from the elastic constants given by Michard *et al.*<sup>12</sup>, and the values of  $n$  are taken from Ref. 13. The various input data for eq. (1) and the resulting value of  $q^*$  are given in Table I.

The values of the effective ionic charge obtained for the nitrates reveal some interesting features. The values are in the range 0.7–1.0. This indicates

that the bonding is essentially ionic. Within the group, however, the effective charge is less for  $\text{Ba}(\text{NO}_3)_2$  than for  $\text{Sr}(\text{NO}_3)_2$  which shows that  $\text{Ba}(\text{NO}_3)_2$  is less ionic than  $\text{Sr}(\text{NO}_3)_2$ . This corroborates the conclusions arrived at by Krishnan<sup>1</sup>. It may be mentioned that a similar trend has been observed<sup>5-10</sup> in the bonding of oxides and sulphides of the alkaline earths.

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## THE POSSIBLE DIENCEPHALIC CONTROL OVER THE PLASMA PROTEINS, PROTEIN BOUND HEXOSE AND SEROMUCOID LEVELS OF THE BLOOD

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### ABSTRACT

The possible role of diencephalic centres in the control of plasma proteins, protein bound hexose and seromucoid levels was studied in the present series. Bilateral electrolytic lesions were made in ventral thalamic and posterior hypothalamic nuclei in 11 cats. Although no significant change in the total plasma proteins was noted in both the series, a significant increase in protein bound hexose in hypothalamic lesioned cats and a general increase in the seromucoid fraction in both the thalamic and hypothalamic lesioned cats was observed. The probable role of neural and neurohumoral influence is discussed.

### INTRODUCTION

THE diencephalic control over the vegetative functions and behaviour is well documented (Hess, 1957). The role of hypothalamic nuclei in

the regulation of levels of plasma proteins was postulated by Lomax based on his experimental and clinical findings (Lomax, 1957 and 1963). Bilateral lesions of zona incerta brought about

increased levels of plasma seromucoid fraction (Mascarenhas *et al.*, 1974). The present study lends support to the hypothesis that destruction of mamillary bodies and ventral thalamic nuclei alter the levels of the plasma protein bound hexose and seromucoids.

#### MATERIALS AND METHODS

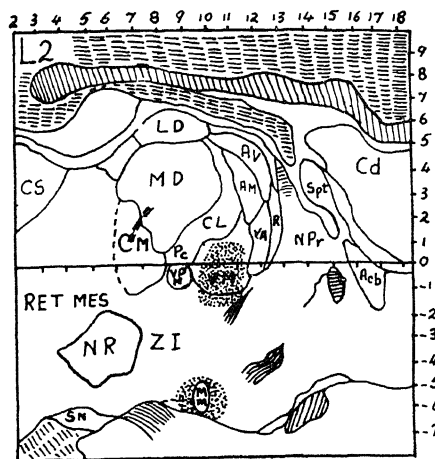
Twelve healthy cats of either sex and of average weight of 2.6 kg. were studied. After capture, they were adequately fed on high protein diet consisting of milk, fish, rice and water. The body weights were checked before and during the subsequent periods after the lesions.

The blood samples were obtained and the plasma separated as per our earlier method (Mascarenhas *et al.*, 1974). The total plasma proteins, protein bound hexose and seromucoid fraction were determined. The estimation of proteins was done by Kjeldahl Nesslerization method (Varley, 1967), of protein bound hexose and of seromucoid fraction in terms of its tryrosine content by the methods modified by Weimer and Moshin (Glick, 1961). All the experimental animals acted as self-controls and the control plasma estimations were done 8 and 15 days after the capture. The cats were then divided into hypothalamic and thalamic series and bilateral electrolytic lesions were made stereotactically in the areas corresponding to left mamillary bodies (F 9, LL 1, H-6) and left ventral thalamic nuclei (F 10-5, LL 2, H-1) respectively as shown in Fig. 1 (Jasper and Ajmone-Marsan, 1960). The lesions in both the series were made through teflon coated unipolar electrode, by passing a DC voltage of about 4 volts (2 milliamperes) for 30 seconds using the rectangular wave stimulator (INCO). Blood samples were collected one hour and 8, 15 and 30 days after the lesions and analysed as before. After 30 days, the animals were sacrificed, the brains removed and fixed in 10% formalin. The sites of the lesions were verified by the standard histological techniques. Figure 1 denotes the diagrammatic representations of the lesions.

#### RESULTS

Table I presents the results statistically computed, obtained in the two series of animals with two different sites of lesions. One cat died before the lesion could be made and hence not included in Table I. It is observed that the total plasma proteins do not show any significant change in both the series following the lesions. The plasma protein bound hexose on the other hand shows a significant increase after lesion in the hypothalamic series whereas the increase in thalamus lesioned cats is not statistically significant. Seromucoid fraction of the plasma is moderately increased in both the

series although the increase is not statistically significant.



Lesions at F<sub>10</sub> to F<sub>11.5</sub>, L<sub>1</sub> to L<sub>2</sub>, H-1 to H-2  
and F<sub>9</sub> to F<sub>11</sub>, L to L<sub>2</sub>, H-5, to H-6

FIG. 1 is the lateral section of the brain at L 2 level (Jasper and Ajmone-Marsan stereotaxic coordinate) and indicates the anteroposterior extent of lesions both in the thalamic and hypothalamic regions.

LD-N, lateralis dorsalis; AV-N, anterior ventralis; MD-N, medialis dorsalis; AM-N, anterior medialis; CL-N, centralis lateralis; VA-N, ventralis anterior; CM-N, centrum medianum; PC-N, paracentralis; VPM-N, ventralis postero-medialis; VM-N, ventralis medialis; ZI, zona incerta, Mm, corpus mamillare.

#### DISCUSSION

The present study suggests that the levels of polysaccharides have been altered by discrete lesions produced in the diencephalic areas. Moderate increase in seromucoid levels after bilateral destruction of zona incerta have been reported earlier in this laboratory (Mascarenhas *et al.*, 1974). The present data suggest that the mamillary bodies and the ventral thalamic neural substrate may influence the synthesis of plasma proteins, but more so of plasma protein bound hexoses and seromucoids. Such a regulatory neural control over the plasma proteins is considered possible by the earlier workers (Lomax, 1957 and 1963; Whurmann and Wunderly, 1960; Freund and Grafe, 1912). It is also reported that a marked depression of circulating antibody was observed after anterior hypothalamic lesion (Tyre and Nalbandev, 1972) and that the rats with hypothalamic lesions have been known to be protected from anaphylaxis (Luparello *et al.*, 1964; Fillip and Szentivanyi, 1958). Histological evidence suggests that the large cells in the mamillary

TABLE I

Showing the alterations in the levels of total plasma protein bound hexose and seromucoid fraction before and after lesions

|                               | Total No. of cats | Total proteins (g) |                         | Protein bound hexose in mg % |                         | Seromucoid fraction in terms of its tyrosine content in mg % |                         |
|-------------------------------|-------------------|--------------------|-------------------------|------------------------------|-------------------------|--|-------------------------|
|                               |                   | Mean control value | Mean value after lesion | Mean control value           | Mean value after lesion | Mean control value   | Mean value after lesion |
| I. Hypothalamic lesioned cats | 7                 | 5.11               | 5.34                    | 178.00                       | 220.41                  | 2.96   | 3.47                    |
|                               |                   | S.D. = $\pm 0.71$  | S.D. = $\pm 0.84$       | S.D. = $\pm 35.09$           | S.D. = $\pm 39.15$      | S.D. = $\pm 0.69$  | S.D. = $\pm 1.09$       |
|                               |                   | S.E. = 0.27        | S.E. = 0.32             | S.E. = 13.26                 | S.E. = 14.00            | S.E. = 0.26  | S.E. = 0.41             |
|                               |                   | $t = 0.555$        |                         | $t = 2.215$                  |                         | $t = 1.03$   |                         |
|                               |                   | $P > 0.10$         |                         | $P < 0.65$                   |                         | $P > 0.10$   |                         |
| II. Thalamic lesioned cats    | 4                 | 3.85               | 3.67                    | 200.18                       | 229.00                  | 3.43   | 5.03                    |
|                               |                   | S.D. = $\pm 0.69$  | S.D. = $\pm 0.48$       | S.D. = $\pm 31.87$           | S.D. = $\pm 50.84$      | S.D. = $\pm 0.62$  | S.D. = $\pm 1.44$       |
|                               |                   | S.E. = 0.33        | S.E. = 0.24             | S.E. = 15.94                 | S.E. = 25.42            | S.E. = 0.31  | S.E. = 0.78             |
|                               |                   | $t = 0.44$         |                         | $t = 1.868$                  |                         | $t = 1.997$  |                         |
|                               |                   | $P > 0.10$         |                         | $P > 0.10$                   |                         | $P < 0.10$   |                         |

bodies are richly supplied with blood and that these cells act as the chemoreceptive mechanism monitoring the plasma albumin concentration (Finley, 1939). It is thus possible that the diencephalic centres might have a neural or neuro-humoral influence on the blood levels of proteins and polysaccharides.

The present two series indicate that the neural substrate exerting an influence on the plasma chemistry may be located in the ventral thalamus involving the ventro medial nucleus and the surrounding nuclei. The area probably extends down to dorsal and ventral posterior hypothalamic nuclei through the zona incerta. This is borne by the fact that the lesions in these areas have hardly altered the levels of plasma proteins but significantly of protein bound hexoses.

Even though the role of other diencephalic centres has not been extensively studied the present work indicates the possible influence of diencephalic neural centres over the synthesis of plasma proteins and the attached polysaccharides and that the control is neural and possibly neuro-humoral in mechanism.

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## RAINFALL FLUCTUATIONS AND CROP YIELDS

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IN recent years, there has been growing interest in possible weather changes and their influence on crop yields. The year 1972-73, during which widespread drought occurred in India and several other regions of the world, is often cited as an important case study.

While the limits of rainy season and consequently the crop growing period in a given region could reasonably be well defined, rainfall fluctuations within the seasons are less definable; statistical probabilities of droughts do not provide for prediction of the behaviour of the current crop season. On the other hand, crop production strategies selected on the basis of performance in the most droughty years might provide built-in safeguards for unforeseen low and/or fluctuating rains encountered during a crop season.

Sorghum popularly known as *Jowar* constitutes the major food crop of the semi-arid tropics almost wholly grown under rainfed conditions. Traditional sorghums grown in black soil areas of the Deccan and Central Indian plateaus are of 5-5½ month duration while the rainy season is of about 100-110 days duration commencing towards the later half of June and terminating before the end of September or middle of October in most parts.

During 1972-73, the long duration traditional sorghums suffered during flowering and thereafter. The *rabi* (October-February) sorghums, which grow and mature primarily on stored profile moisture, either could not be sown in time or failed to yield for want of adequate reserve soil moisture. However, the early maturing hybrids and high yielding varieties of sorghum of 95-110 days duration, as against 150-day locals, when planted in time, and well managed, gave satisfactory yields during *kharif*. Similarly an early maturing hybrid CSH-1, when planted earlier in *rabi* season under proper management, also returned very satisfactory yields. It appears, therefore, that years like 1972-73 need not create a scare for rainfed agriculture and a choice of suitable genotypes, together with appropriate management practices, could still return average yields several times higher than all-India averages which are presently very low for most rainfed crops. The purpose of this paper is to analyse the rainfall-yield relationships of some superior sorghum 'hybrids' and 'varieties' grown under good management, during 1972-73 *kharif* and *rabi* seasons with focus on the controllable and manage-

able part of the variation in yield so as to realise satisfactory agricultural yields in spite of rainfall fluctuations.

## 1972-73 KHARIF SEASON

Multiple regression analysis utilising: (1) total rainfall during the year, (2) the number of rainy days and (3) the coefficient of variation in the monthly rainfall at each of the locations, as auxiliary variates, was attempted to study the relationship between rainfall attributes and grain yields. The set of 29 locations, from which data were available provided a reasonably representative sample of the environments, in which the sorghum crop is being grown and will continue to be grown in the country. The regression analysis has been carried out separately, for each of the high yielding hybrids and varieties. In the case of hybrids and varieties, where regression coefficients associated with rainfall attributes were significant, the coefficients have been recalculated, after deleting the less important auxiliary variates. If the proportion of variation attributable to the rainfall characteristics is small, the capacity of the genotype to withstand drought will be greater.

The mean yields, the total rainfall, the number of rainy days and the coefficient of variation in the monthly rainfall are presented in Table I. The analysis of variance and the values of the regression coefficients are presented in Tables II and III, respectively.

The genotypes 302 and 604 among the varieties, and CSH-1, CSH-3 and CSH-5 among the hybrids seem to be less dependent on the vagaries of rain as compared with the rest of the varieties and hybrids, *Swarna*, CS-3541, CSH-2 and CSH-4. The contributions of the number of rainy days ( $x_2$ ) and the coefficient of variation in the monthly rainfall ( $x_3$ ) to the variation in the yield of sorghum is larger than that of total rainfall ( $x_1$ ) as could be seen from the significant values of  $b_2$  and/or  $b_3$  in the case of *Swarna*, CS-3541, CSH-2 and CSH-4. In other words, the analysis suggests that yield of sorghum is influenced more by the number of rainy days and the distribution of monthly rainfall rather than the total rainfall. The negative values of  $b_3$  or  $b_3'$  indicate that larger values of coefficients of variation adversely affect yields.

The percentage of variation, ascribable to rainfall attributes, is shown in Table IV. The amount of

TABLE I

Mean yields (kg/ha) of varieties and hybrids during 1972-73 kharif, total rainfall, number of rainy days and coefficient of variation(%) in the monthly rainfall

| Sl. No. | Location      | Varieties |         |      |      | S.E. <sub>m</sub> (kg/ha) | Hybrids |       |       |       |       | S.E. <sub>m</sub> (kg/ha) | Total rainfall mm (x <sub>1</sub> ) | No. of rainy days (x <sub>2</sub> ) | c.v. (%) of monthly rainfall (x <sub>3</sub> ) |
|---------|---------------|-----------|---------|------|------|---------------------------|---------|-------|-------|-------|-------|---------------------------|-------------------------------------|-------------------------------------|--|
|         |               | Swarna    | CS 3541 | 302  | 604  |                           | CSH-1   | CSH-2 | CSH-3 | CSH-4 | CSH-5 |                           |                                     |                                     |  |
| 1       | Parbhani      | 4592      | 3033    | 6326 | 3322 | 239                       | 5701    | 4163  | 4133  | 5550  | 5171  | 342                       | 524.9                               | 44                                  | 126  |
| 2       | Jalgaon       | 3322      | 3032    | 3148 | 3811 | 353                       | 2621    | 3040  | 3471  | 2994  | 3699  | 425                       | 650.0                               | 38                                  | 135  |
| 3       | Karad         | 3179      | 3640    | 2635 | 2345 | 313                       | 4316    | 4053  | 3662  | 4374  | 4233  | 309                       | 367.8                               | 55                                  | 152  |
| 4       | Kolhapur      | 3356      | 3356    | 3761 | 4398 | 215                       | 3868    | 5102  | 4115  | 3292  | 4444  | 446                       | 439.4                               | 63                                  | 147  |
| 5       | Buldhana      | 2511      | 2751    | 3796 | 2426 | 398                       | 3860    | 1855  | 3486  | 4068  | 4754  | 440                       | 463.4                               | 35                                  | 148  |
| 6       | Nagpur        | 3329      | 3570    | 4398 | 3358 | 283                       | 3309    | 2551  | 2990  | 3309  | 3022  | 262                       | 546.4                               | 54                                  | 147  |
| 7       | Yectmal       | 3655      | 3402    | 4349 | 2619 | 128                       | 4862    | 3593  | 3521  | 4994  | 4860  | 308                       | 699.1                               | 59                                  | 129  |
| 8       | Dharwar       | 4467      | 3378    | 3176 | 3880 | 397                       | 6057    | 5400  | 5615  | 4995  | 6737  | 448                       | 722.44                              | 73                                  | 97   |
| 9       | Bagalkot      | 1555      | 1540    | 1514 | 1808 | 168                       | 2125    | 1052  | 773   | 2073  | 2335  | 185                       | 491.5                               | 40                                  | 113  |
| 10      | Arbhavi       | 3543      | 3271    | 3938 | 3210 | 295                       | 4230    | 4576  | 3934  | 4181  | 4675  | 326                       | 429.2                               | 32                                  | 99   |
| 11      | Gangavati     | 5069      | 3102    | 5683 | 5752 | 420                       | 5425    | 5426  | 6545  | 7346  | 6340  | 477                       | 313.5                               | 41                                  | 114  |
| 12      | Rajendranagar | 4485      | 4898    | 5142 | 3079 | 279                       | 5023    | 2421  | 4107  | 4902  | 5185  | 445                       | 419.9                               | 36                                  | 102  |
| 13      | Anantapur     | 674       | 75      | 447  | 1075 | 1151                      | 1037    | 1057  | 800   | 810   | 706   | 157                       | 557.3                               | 57                                  | 138  |
| 14      | Yemmiganur    | 3746      | 2439    | 3573 | 5576 | 331                       | 7201    | 4688  | 5374  | 6574  | 9029  | 833                       | 394.4                               | 27                                  | 100  |
| 15      | Navsari       | 4080      | 3358    | 3857 | 3065 | 266                       | 4166    | 3663  | 4300  | 2650  | 5521  | 281                       | 963.9                               | 59                                  | 172  |
| 16      | Surat         | 2095      | 2196    | 2111 | 1973 | 233                       | 2200    | 2097  | 2353  | 2395  | 2727  | 226                       | 791.7                               | 35                                  | 173  |
| 17      | Deesa         | 1828      | 818     | 1147 | 444  | 240                       | 2104    | 1963  | 2629  | 2172  | 1622  | 239                       | 366.0                               | 18                                  | 205  |
| 18      | Gwalior       | 1943      | 2978    | 2886 | 2587 | 259                       | 4831    | 2264  | 3651  | 282   | 4624  | 426                       | 864.0                               | 31                                  | 234  |
| 19      | Khargaoan     | 1974      | 2110    | 1721 | 1936 | 265                       | 1691    | 1535  | 1217  | 2547  | 2412  | 230                       | 392.5                               | 33                                  | 164  |
| 20      | Chindwara     | 2731      | 3339    | 2951 | 243  | 309                       | 3251    | 1284  | 2609  | 3539  | 3284  | 420                       | 671.1                               | 37                                  | 209  |
| 21      | Indore        | 4492      | 3620    | 5021 | 3486 | 312                       | 5860    | 5464  | 5788  | 5942  | 7144  | 383                       | 679.0                               | 52                                  | 216  |
| 22      | Powerkheda    | 1594      | 2025    | 1661 | 2523 | 150                       | 2942    | 2260  | 2592  | 2707  | 3109  | 245                       | 908.4                               | 44                                  | 226  |
| 23      | Coimbatore    | 4010      | 3847    | 2551 | 5166 | 320                       | 4736    | 5218  | 4792  | 4361  | 3347  | 408                       | 962.0                               | 48                                  | 127  |
| 24      | Bhavanisagar  | 4525      | 3390    | 3760 | 3258 | 243                       | 4816    | 5334  | 5405  | 4959  | 6219  | 300                       | 1384.2                              | 49                                  | 160  |
| 25      | Tindivanam    | 1910      | 1426    | 973  | 957  | 202                       | 2571    | 2278  | 2194  | 2998  | 2386  | 307                       | 839.2                               | 46                                  | 131  |
| 26      | Pudukottai    | 4339      | 3753    | 4305 | 3108 | 160                       | 5304    | 5147  | 5476  | 5500  | 5342  | 276                       | 1014.0                              | 58                                  | 102  |
| 27      | Vallabhnagar  | 3330      | 2555    | 3879 | 2480 | 293                       | 3018    | 2405  | 2416  | 2338  | 2778  | 352                       | 311.8                               | 27                                  | 166  |
| 28      | Jhansi        | 2693      | 2372    | 2519 | 2694 | 212                       | 2921    | 2158  | 1677  | 1252  | 3582  | 291                       | 743.5                               | 31                                  | 225  |
| 29      | Kanpur        | 822       | 864     | 598  | 1478 | 144                       | 2665    | 1456  | 2224  | 2050  | 2286  | 227                       | 475.4                               | 27                                  | 182  |
|         | Mean          | 3098      | 2765    | 3166 | 2830 | 130                       | 3886    | 3224  | 3530  | 3710  | 4268  | 111                       | 634.0                               | 42.4                                | 153.1  |

TABLE II  
Regression analysis

| Source                    | d.f. | Mean sum of squares |          |                 |                 |                 |          |                 |         |                 |
|---------------------------|------|---------------------|----------|-----------------|-----------------|-----------------|----------|-----------------|---------|-----------------|
|                           |      | Varieties           |          |                 |                 | Hybrids         |          |                 |         |                 |
|                           |      | Swarna              | CS 3541  | 302             | 604             | CSH-1           | CSH-2    | CHS-3           | CSH-4   | CSH-5           |
| Regression                | 3    | 4568929*            | 2763627* | 3619974<br>(NS) | 4049214<br>(NS) | 4327697<br>(NS) | 8597944† | 5772548<br>(NS) | 641259* | 5653567<br>(NS) |
| Deviation from regression | 25   | 1106086             | 915198   | 2085786         | 1580066         | 1983491         | 1595692  | 1953846         | 2102004 | 3793649         |

\* Significant at 5% level.

† Significant at 1%.

NS=Not significant.

TABLE III  
Regression coefficients

| Regression coefficient | Varieties     |               |               |                | Hybrids       |                |               |               |                |
|------------------------|---------------|---------------|---------------|----------------|---------------|----------------|---------------|---------------|----------------|
|                        | Swarna        | CS 3541       | 302           | 604            | CSH-1         | CSH-2          | CSH-3         | CSH-4         | CSH-5          |
| $b_1$                  | -0.51<br>(NS) | 0.11<br>(NS)  | -0.88<br>(NS) | 0.05<br>(NS)   | 0.40<br>(NS)  | 0.72<br>(NS)   | 0.84<br>(NS)  | -0.05<br>(NS) | 0.50<br>(NS)   |
| $b_2$                  | 42.83*        | 57.91*        | 38.99<br>(NS) | 22.73<br>(NS)  | 32.79<br>(NS) | 53.17*         | 39.16<br>(NS) | 28.73<br>(NS) | 31.12<br>(NS)  |
| $b_3$                  | -7.69<br>(NS) | -2.88<br>(NS) | -6.35<br>(NS) | -12.17<br>(NS) | -9.53<br>(NS) | -10.21<br>(NS) | -9.36<br>(NS) | -15.33*       | -12.86<br>(NS) |
| $b_2'$                 | 46.09†        | 41.31†        | ..            | ..             | ..            | 68.25†         | ..            | ..            | ..             |
| $b_3'$                 | ..            | ..            | ..            | ..             | ..            | ..             | ..            | -18.02†       | ..             |

\* Significant at 5% level. † Significant at 1% level. NS=Not significant.

Note:  $b_1$ ,  $b_2$  and  $b_3$  represent the coefficients of regression associated with total rainfall, number of rainy days, and the coefficient of variation (c.v.%) in the monthly rainfall respectively.

$b_2'$  Coefficient of regression associated with the number of rainy days after deletion of the total rainfall and coefficient of variation in the monthly rainfall.

$b_3'$  Coefficient of regression associated with coefficient of variation in the monthly rainfall after deletion of total rainfall and number of rainy days.

TABLE IV  
Percentage of variation accounted for by regression

| Percentage of variation                            | Varieties |         |      |      | Hybrids |       |       |       |       |
|--|-----------|---------|------|------|---------|-------|-------|-------|-------|
|  | Swarna    | CS 3541 | 302  | 604  | CSH-1   | CSH-2 | CSH-3 | CSH-4 | CSH-5 |
| With variables $x_1$ , $x_2$ and $x_3$             | 33.1      | 26.6    | 17.2 | 23.5 | 20.7    | 39.3  | 26.2  | 26.9  | 15.2  |
| With $x_2$ alone (after deleting $x_1$ and $x_3$ ) | 23.8      | 25.4    | ..   | ..   | ..      | 32.9  | ..    | ..    | ..    |
| With $x_3$ alone (after deleting $x_1$ and $x_2$ ) | ..        | ..      | ..   | ..   | ..      | ..    | ..    | 22.2  | ..    |

Note:  $x_1$ =Total rainfall,  $x_2$ =Number of rainy days,  $x_3$ =The coefficient of variation in the monthly rainfall (c.v. %).

variation in yield ascribable to rainfall is mostly determined by the number of rainy days and/or the coefficient of variation in the monthly rainfall within the limits of rainfall received. What is more important is that in none of the cases studied, the variation ascribable to rainfall characteristics exceeds 40% of the total variation. This shows that still a large proportion of the total variation in yield, upto 85% over the locations, is attributable to causes other than fluctuations in rainfall as discussed later.

#### 1972-73 RABI SEASON

In view of the low rainfall received during the early months of June, July and August and the anticipated low production of sorghum during kharif season, it was thought that possibilities of compensating for kharif losses may be explored in the rabi sorghum belt.

The low rainfall preceding the rabi season resulted in low levels of profile moisture which was apt to adversely influence the rabi production. Keeping

this in mind a rabi Jowar production campaign based on the following principles was suggested: (1) Advancing dates of planting well into early September, without waiting for the traditional sowing dates in October so that the crop has a chance of getting some rain during early growth period; (2) Growing early maturing 100-day hybrids in place of 120-135 day locals; (3) All basal fertilization; (4) Full crop stands and (5) Seed treatment with a chemical, carbofuran, to prevent damage by sorghum shoot fly, a seedling pest. The whole programme was oriented towards earliness, so that the crop was raised during the period September-mid December instead of the traditional October-February.

A well directed programme of this type in one District of Andhra Pradesh, Khammam, over about 50,000 acres undertaken by the Department of Agriculture was an unqualified success. This was partly due to the somewhat favourable rains received during early crop growth period. While weather

TABLE V

Number of crop cutting experiments and mean grain yield in different Samithies of Khammam District, Andhra Pradesh—Rabi 1972-73

| S. No. | Samithi         | No. of experiments conducted |       | Grain yield (kg/ha) |       | % over local |
|--------|-----------------|------------------------------|-------|---------------------|-------|--------------|
|        |                 | CSH-1                        | Local | CSH-1               | Local |              |
| 1.     | Wyra            | 3                            | 3     | 449                 | 1318  | 340          |
| 2.     | Khammam         | 3                            | 3     | 4983                | 2109  | 236          |
| 3.     | Burgampad       | 2                            | ..    | 4457                | ..    | ..           |
| 4.     | Kothagudam      | 6                            | 6     | 4915                | 1233  | 398          |
| 5.     | Yellandu        | 5                            | 4     | 2683                | 1297  | 207          |
| 6.     | Bhadrachalam    | 1                            | 1     | 4571                | 1186  | 385          |
| 7.     | Tirumalayapalem | 8                            | 4     | 4235                | 1742  | 243          |
|        | Mean            |                              |       | 4239                | 1477  | 287          |

analysis of this region is not available, yield data obtained by the Department of Agriculture from crop cutting experiments (Table V); speak for the efficacy of the programme.

Besides, there is considerable experimental evidence accumulated over years in the *rabi* Jowar belt, that advancing dates of planting, during *rabi* enables certain genotypes (varieties or hybrids) respond better to higher seed rates and all basal fertilization, thereby, providing insurance against drought and also result in much higher levels of production.

#### PLANNING FOR RAINFED AGRICULTURE

Rainfall analysis by itself does not provide for prediction about the behaviour of the immediate cropping season. On the other hand, analyses based on more successful experiments and profitable farmer experience during the worst years encountered is more positive and could provide for development of cropping plans which could be least risky if the rains are subnormal and highly profitable if rains are normal. The current analysis is an attempt in this direction.

Genotype alterations involving reduction of duration, reduction of total dry matter and its more efficient distribution between stalk and ear (economic product), adjustments in times of sowing, maintenance of optimum plant populations, use of fertilizer, plant protection measures and practice of suitable systems of cropping, ratooning, etc., all of which are in the realm of human control could enhance productivity levels and impart stability to production in rainfed agriculture. During 1972-73, sorghum yield levels in well managed experiments

and with several farmers were of the order of 25-30 q/ha against the national average of 5 q/ha in normal years and near total failure during 1972-73.

A point of significance in planning for rainfed agriculture emerges here. Attempts at enhancing agricultural productivity do realise the role of environmental barriers and the need to transgress 'error' (environmental) limits. Yet, the targetted yield increases planned more particularly for rainfed agriculture are frequently within the limits of environmental error and do not provide for the much needed increments of a larger magnitude. A breakthrough in rainfed agriculture can, therefore, be expected only by planning for large quantum jumps rather than for slow and graded annual targets which are within the realm of environmental fluctuations. This approach may all the more be necessary, in the initial years since the present all-India averages for most rainfed crops are less than 500 kg/ha and the available know-how has the potential to elevate the national averages several fold.

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## LETTERS TO THE EDITOR

THE CRYSTAL STRUCTURE OF A 2:1  
COMPLEX BETWEEN PHENYLBUTAZONE  
AND PIPERAZINE

THE crystal structure of the title compound has been determined as part of a programme of systematic single crystal x-ray investigations on non-narcotic analgesics and their crystalline complexes<sup>1-4</sup>.

Large yellowish crystals of the complex were obtained by slow evaporation at room temperature of a solution of the components in methanol. The space group and unit cell dimensions of the complex were determined from oscillation and Weissenberg photographs. The density, measured by floatation method in a mixture of carbon tetrachloride and benzene, indicated the presence of four phenylbutazone and two piperazine molecules in the unit cell. The composition was confirmed by chemical microanalysis and the subsequent structure solution. The crystal data are summarized below.

Space group  $P2_1/c$

$a = 8.04 \pm 0.02$ ,  $b = 15.06 \pm 0.02$ ,  $c = 15.60 \pm 0.02$  Å,  $\beta = 95.2 \pm 0.5^\circ$ ;  $V = 1878.7 \text{ Å}^3$ ;  
 $D_m = 1.238 \pm 0.005$ ,  $D_c = 1.238 \text{ gm/cc}$ .

Three-dimensional x-ray data were recorded on multiple film equi-inclination Weissenberg photographs using  $\text{CuK}\alpha$  radiation for reciprocal levels  $h\text{Kl}$ ,  $L = 0$  through 13 and  $h\text{kl}$ ,  $K = 0$  and 1. The intensities were estimated visually. The structure was determined by the direct methods followed by conventional Fourier techniques. The atomic parameters were refined isotropically by the structure factor least squares procedure to a current  $R$  value of 0.175 for 2395 observed reflexions. The structure, as viewed along the  $a$ -axis, is shown in Fig. 1. Further refinement of the structure is in progress.

The asymmetric unit of the crystal contains one molecule of phenylbutazone and half a molecule of piperazine. The phenylbutazone molecule exists as a negative ion on account of the deprotonation of the ring carbon atom C 4, consequent to the formation of the complex. The deprotonation referred to above leads to significant structural differences between the anionic phenylbutazone molecule in the complex and the neutral molecules in the crystals of phenylbutazone<sup>4</sup>. In the latter, the carbon atom C 4 is tetrahedral whereas in the former, it is

trigonal, resulting in different orientations of the butyl group in the two cases. Also, in neutral phenylbutazone molecules, the two C—O bonds are double and the two C—O bonds in the pyrazolidine ring are single. In the anionic molecule in the complex, however, the excess electron is delocalized over these four bonds, giving them partial double bond character. The centrosymmetric piperazine molecule in the structure, with both the nitrogen atoms protonated, is a doubly charged cation. The molecule is in the chair form with nitrogen atoms at the two apices. The crystal structure is stabilized by a three-dimensional arrangement of N—H...O hydrogen bonds with the nitrogen atoms in piperazine as donors and the carbonyl oxygens in phenylbutazone as acceptors.

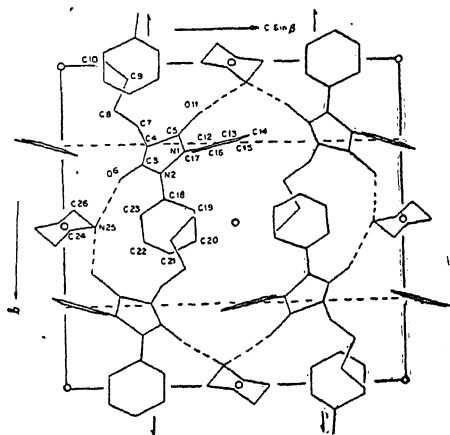


Fig. 1. The structure as viewed along the  $a$ -axis.

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### INTRAMOLECULAR DECARBOXYLATION OF 3-(4'-ETHOXYPHENYL)-GLUTACONIC ANHYDRIDE: FORMATION OF A KETENE DIMER

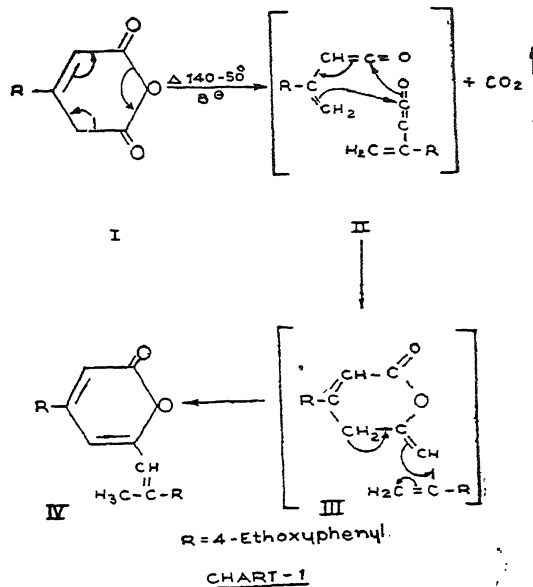
THE condensation of phthalic anhydride with acetic anhydride in presence of potassium acetate has been reported to give phthalylacetic acid<sup>1</sup>. 3-(4'-Ethoxyphenyl)-glutaconic anhydride, under similar conditions behaved in an analogous manner and furnished 3-(4'-ethoxyphenyl)-glutaconylacetic acid<sup>2,3</sup>. Perkin-type reaction between phthalic anhydride and phenylacetic acid in presence of sodium acetate gave benzalphthalide<sup>4,5</sup>. Similar extension of the reaction to 3-(4'-ethoxyphenyl)-glutaconic anhydride (I), however, afforded a product, namely, 4-(4'-ethoxyphenyl)-6-[ $\beta$ -(4'-ethoxyphenyl)-propenyl]-2-pyrone (IV) to the exclusion of expected (V). The formation of (IV) was confirmed on the basis of elemental analysis, oxidation studies and IR spectral determination.

The elemental analysis of the compound was in agreement with structure (IV). Alkaline permanganate oxidation of the product yielded *p*-ethoxybenzoic acid (m.p. 193-95°) and oxalic acid. The IR spectrum of the product exhibited bands at 1718, 1610, 1530, 1512, 1180, 1030 and 840  $\text{cm}^{-1}$  characteristic of compounds of type (IV)<sup>6,7</sup>. The formation of (IV), therefore, suggests that anhydride (I) in presence of a base like sodium acetate undergoes intramolecular decarboxylation and the resulting ketene (II) dimerizes to (III) which subsequently yields pyrone (IV)<sup>8</sup>. The plausible mechanism entailing the formation of (IV) is represented in Chart 1.

#### Experimental

4-(4'-Ethoxyphenyl)-6-[ $\beta$ -(4'-ethoxyphenyl)-propenyl]-2-pyrone (IV): A mixture of 3-(4'-ethoxyphenyl)-glutaconic anhydride (I, 2.32 g), phenylacetic acid (1.36 g) and fused sodium acetate (1.64 g) was heated in an oil bath at 140-150° for 4 hr. Initially the mixture liquified and then rapid evolution of carbon dioxide occurred giving reddish brown coloured viscous liquid. The mixture was then cooled to room temperature and

treated with aqueous bicarbonate. The residue thus left was thoroughly washed with water and crystallized from 50% ethanol, yielding pale yellow coloured crystals, m.p. 150-52° (Anal. Calcd. for  $\text{C}_{24}\text{H}_{24}\text{O}_4$ : C, 76.59; H, 6.38%. Found: C, 76.48, H, 6.44%.)



We wish to thank Dr. S. S. Karmarkar, Bombay, for his interest in the work and to Professor S. C. Bhattacharya, Indian Institute of Technology, Bombay, for IR spectral determination.

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### CONTROL OF *FUSARIUM* WILT OF PIGEON PEA WITH BAVISTIN, A SYSTEMIC FUNGICIDE

UNTIL recently, no good chemical control measure was known for most of the vascular wilt diseases. However, with the advent of systemic fungicides, particularly those of the benzimidazole group, successful chemical control of vascular wilt diseases of some plants has been achieved in the recent years. Wilt disease due to *Fusarium oxysporum* f. sp. *udum* (Butler) Snyder and Hansen causes considerable damage to the pigeon-pea crop. Only recently, some success in control has been reported from this laboratory with the use of Benlate, a systemic fungicide of bezimidazole group<sup>1</sup>. Results of some further studies on the control of pigeon pea wilt by using Bavistin (2 Methyl-2-benzimidazole carbamate, 50% w/w), a broad spectrum systemic fungicide of the same group, are briefly reported here.

Plants raised in garden soil mixed with farmyard manure in 6 in. pots were inoculated when 3 weeks old. Inoculum was prepared by mixing 10–12 days old culture grown on sand maize-meal medium at 28°C with equal amount of dry, sterilized soil. Top soil in each pot to be inoculated was replaced

with 100 g of inoculum which was then covered with a thin layer of removed top soil. Treatments with the fungicide, used always as a suspension in water, were given mostly as soil drench, rarely as spray, on different dates in relation to the time of inoculation. For each treatment, there were 4 pots, each with 3–5 plants. At different intervals after inoculation, the number of plants showing wilt symptoms were recorded and the degree of wilting shown by the individual leaves was assessed on a 0–4 scale. The total of these values for a plant, when divided by its number of leaves, gave the disease index on leaf basis.

In the first experiment, there were four treatments in addition to the uninoculated control and inoculated control series. The fungicide was applied at different concentrations, in 50 ml portions to each pot as soil drench, 10 days before, and 5 and 22 days after inoculation, that is well ahead of, soon after and at a late stage of infection. While the concentration of 4,000 ppm was used on all the three occasions, a lower concentration of 2,000 ppm was also used when the treatment was given soon after inoculation.

It appears from the summarized results (Table I) that half of the 20 plants in the inoculated control series developed symptoms within 3 weeks and 7 more within the next 3 weeks and all these plants died within the experimental period (11 weeks). The remaining 3 plants did not develop any symptoms. In contrast to this, soil drench with 4,000 ppm Bavistin 10 days before inoculation gave the treated plants total protection against the disease. None

TABLE I

*Effect of Bavistin, applied as soil drench, on the incidence and severity of Fusarium wilt in pigeon pea plants*

| Treatment  | No. of plants | Disease incidence<br>(% of plants affected) |      |      | Mean wilt index<br>per leaf |      |      |
|--|---------------|---|------|------|-----------------------------|------|------|
|  |               | Days after inoculation                      |      |      | Days after inoculation      |      |      |
|  |               | 21  | 42   | 77   | 21                          | 42   | 77   |
| Uninoculated   | 16            | 0   | 0    | 0    | 0                           | 0    | 0    |
| Inoculated (control)   | 20            | 50.0  | 85.0 | 85.0 | 0.86                        | 1.95 | 3.18 |
| Inoculated + 4,000 ppm<br>Bavistin (22 days after<br>inoculation)  | 17            | 23.5  | 29.4 | 29.4 | 0.67                        | 0.63 | 0.48 |
| Inoculated + 2,000 ppm<br>Bavistin (5 days after<br>inoculation)   | 16            | 6.3   | 6.3  | 6.3  | 0.09                        | 0.16 | 0.16 |
| Inoculated + 4,000 ppm<br>Bavistin (5 days after<br>inoculation)   | 16            | 6.3   | 6.3  | 6.3  | 0.09                        | 0.09 | 0.08 |
| Inoculated + 4,000 ppm<br>Bavistin (10 days before<br>inoculation) | 17            | 0   | 0    | 0    | 0                           | 0    | 0    |
| C.D. (5%)  |               |   |      |      | 0.22                        | 0.24 | 0.18 |
| C.D. (1%)  |               |   |      |      | 0.31                        | 0.33 | 0.25 |

of them developed even minor symptoms. Similar treatments with 2,000 and 4,000 ppm suspensions only 5 days after inoculation were also highly effective. Only one out of 16 plants in each of the two treatments showed signs of infection, but the wilt symptoms did not progress much beyond the lower leaves and both the plants recovered. Even the late treatment with 4,000 ppm Bavistin, given 22 days after inoculation, gave significantly effective control. Only 4 out of 17 plants developed symptoms within 3 weeks and one more became affected in the succeeding weeks. Of the affected plants, only 2 died and the rest recovered. The results were confirmed by repeating the experiment.

In the second experiment, a few additional treatments were included. The fungicide was also applied as soil drench at 2,000 ppm, 10 days before inoculation, and as foliar spray at 2,000 and 10,000 ppm, only 2 days after inoculation. About 20 ml of suspension was used as spray to give good coverage to both the leaf surfaces of all the plants in a pot. Obviously, a small portion of it dripped from the leaves into the top soil. Symptom appearance was slow in this experiment. Only 20% of the plants in the inoculated control series developed symptoms within 5 weeks. In the course of the next 6 weeks, a total of 60% of the plants became affected and the mean disease index changed from 0.8 to 2.0. Pre-inoculation drench treatment with 2,000 ppm suspension gave the plants total protection against the disease. When used as foliar spray, the fungicide gave total protection at 10,000 ppm. At 2,000 ppm, however, a small proportion (17%) of plants developed symptoms by the end of fifth week and the percentage of affected plants increased to 34% in course of the next 6 weeks. Only one of them recovered; all others died.

Bavistin was also tested for its toxicity against the pathogen, using poisoned food technique, at 1,000, 100, 10 and 1 ppm in PDA medium. There were four replicates for each treatment. Radial growth was recorded after 5 and 7 days' incubation at 28° C. Bavistin inhibited growth totally at 1,000 and 100 ppm and significantly even at 10 ppm. At 1 ppm, the fungicide had no effect.

Observations clearly indicate that Bavistin holds great promise as a fungicide in the control of pigeon pea wilt. If adequately absorbed by the plant, Bavistin may not only prevent infection from taking place but may also cure the established infection, if it has not already progressed much.

Bavistin used in this study was obtained through the courtesy of BASF, India Ltd., Bombay, India.

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#### A NEW RECORD OF PARASITIC DODDER ON CHICKPEA (*CICER ARIETINUM* L.)

A FIELD of chickpea near village Mana, Raipur (India) was severely attacked by dodder (*Cuscuta* sp.), a phanerogamic plant parasite (Fig. 1). The chickpea plants were badly entwined with slender vines of *Cuscuta* and the parasitized plants dried prematurely and produced a few small shrivelled seeds. Transverse sections of the host stems showed the presence of haustoria of the parasite.



FIG. 1. Dodder (*Cuscuta hyalina* Roth.) on chickpea.

The parasite was leafless, filiform, and its vines were more slender than *Cuscuta reflexa* Roxb. which formed dense yellow masses on the host plants. Flowers were pale yellow, bracteate, tetra- or pentamerous and found in clusters. Corolla scales were absent. Stamens exerted between the corolla lobes; styles 2, unequal, filiform; stigmas capitate; seeds four, brownish, triangular,



The parasite has been tentatively identified as *Cuscuta hyalina* Roth. based on the description given by Hooker (1885). This is first record of this dodder parasitising chickpea plants in nature in India.

The specimens have been deposited in the Herbarium of the Department of Plant Pathology, J.N. Agricultural University, Jabalpur 482 004, India.

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Jawaharlal Nehru Krishi Vishwa L. K. JOSHI.

Vidyalaya,  
Jabalpur 482 004, May 19, 1975.

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#### RADIATION DAMAGE OF P-32 AND ADENOPITUITARY

IONIZING radiations influence the anterior pituitary in 2 different ways: (i) direct effect and (ii) indirect effect through hypothalamus, thyroid, adrenal, and gonads. A combination of both these effects will be revealed after a certain time of irradiation.

The histopathological slides of the pituitary gland prepared from P-32 injected mice always show the ventral margin of the adenopituitary containing a greater number of dead cells, pyknotic nuclei with shrunken cytoplasm, etc. The ventral margin of the adenopituitary consists more of basophils as compared to the acidophils than the rest of the pituitary<sup>1</sup>. Our impression is that the basophils are comparatively less susceptible to radiation than the acidophil cells<sup>2</sup>. These observations were made on Swiss albino mice during development stages of the pituitary. P-32 was injected to young animals at the rate of  $1.0 \mu \text{Ci/g}$  body weight at various intervals after parturition and sacrificed regularly at weekly intervals.

It appears that the damage done to the pituitary by the above dose of radiophosphorus by direct and indirect ways only, cannot account for its chosen ventral margin lesions but also for the direct hitting of the ventral margin cells by radiophosphorus which are accumulated in the surrounding floor bones of the cranium, particularly the sella, which remains in close contact with the adenopituitary. In general, bones show the greatest avidity for this metabolite. P-32 has a maximum energy of 1.69 Mev. and a maximum range of 7 mm in the soft tissue. To confirm this, adult mice were injected with P-32 at the dose rate of 2, 3, and  $5 \mu \text{Ci/g}$  body weight. The results were in conformity with the observations made on the young animals, i.e., a

greater number of cell death towards the ventral margin of the adenopituitary than the rest of it.

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University of Rajasthan,  
Jaipur 302 004, April, 22, 1975.

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#### GYNANDROMORPHS OF *CULEX FATIGANS*— THE FILARIAL MOSQUITO

GYNANDROMORPHS have been found in fair numbers in the laboratory strains of Culicine mosquitoes. More than half the known mosquito gynandromorphs have occurred in the genus *Culex*. Both *Culex pipiens* (Laven, 1967)<sup>1</sup> and *Aedes aegypti* (Craig and Hickey, 1967)<sup>2</sup> have been specially known for the large number of naturally occurring gynandromorphs. Kitzmiller (1953)<sup>3</sup> listed five *Culex* species with reported gynandromorphs. Subsequent references to gynandromorphs include those of Brust (1966)<sup>4</sup> and Lee (1967)<sup>5</sup>. Since then two more species have been added to the list: *Culex tritaeniorhynchus* (Aslamkhan and Baker, 1969)<sup>6</sup> and *Culex fuscocephalus* (Aslamkhan, 1970)<sup>7</sup>.

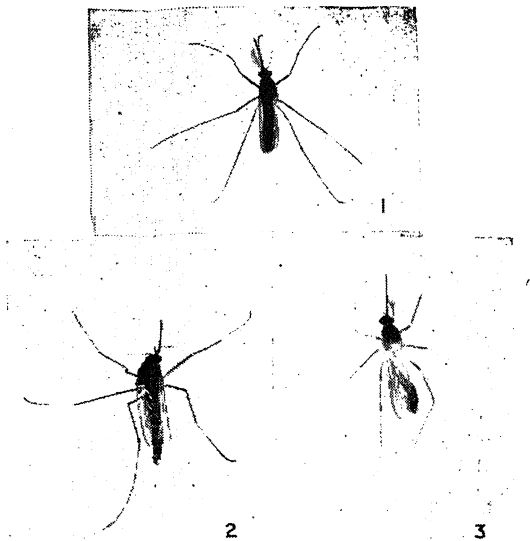
The gynandromorphs reported here were discovered in inbred strains of *Culex fatigans* during a screening programme for mutant genes. The gynandromorphs were of three broad types:

(a) *Anterior-posterior gynander* (Fig. 1).—In this case, the anterior region was phenotypically male; head with antennae and palpi typical of a male. The posterior region was typically female; with wings of the female type and abdomen with well developed ovaries. Fore and mid pairs of appendages were typically like those of a female.

(b) *Anterior-posterior gynander* (Fig. 2).—In this case, the anterior region was phenotypically female and the posterior region male. The mouth parts were typically like those of a female; the abdomen, hypopygium and the wings resembled those of a male. However, the appendages exhibited bilateral characters. The fore and mid appendages of the right side were female-like and the corresponding ones of the left side were male-like. Testes were well developed.

(c) *Bilateral-gynander* (Fig. 3).—The right side of the body resembled a male and the left side a female. The antenna, appendages 1 and 2, abdomen and hypopygium, all of the right side resembled those of a typical male. The palp of the right side was abnormal, being intermediate between that of a male and a female. The antenna, palp, appendages 1 and 2 and abdomen, all of the

left side resembled those of a typical female. Thus, the right side of the mosquito was male-like with well developed testis and left side female-like with well developed ovary.



FIGS. 1-3. Fig. 1. Anterior-posterior gynander : anterior male and posterior female. Fig. 2. Anterior-posterior gynander : anterior female and posterior male. Fig. 3. Bilateral gynander : right side male and left side female.

While the mouth parts in the first type were not adapted for sucking the blood, they were adapted for the same in the second type. However, as the abdomen in the second type was typically male-like in not being adapted for holding the blood, the abdomen broke open and as a result the gynander died. Hence, the gynanders of these two types as well as the third type failed to lay eggs.

The causes for the high frequency of gynandromorphs in mosquitoes are not understood. Gilchrist and Haldane (1947)<sup>8</sup>, Laven (1957)<sup>9</sup>, Rai and Craig (1963)<sup>10</sup> and Craig and Hickey (1967)<sup>2</sup> have suggested mechanisms for the origin of gynandromorphs in Culicine mosquitoes. Based on the evidence from genetic markers, they suggest that there is a hereditary tendency, but no simple Mendelian gene seems to be involved. It is interesting that all the gynandromorphs described here were isolated from only one strain (Bangalore-Challaghatta tank). The occurrence of these gynanders in a particular strain and their appearance in a few generations could be the result of some genetic factors whose exact nature is as yet unknown.

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#### RECORD OF *SPIRONOURA BREVISPICULATA* BAYLIS, 1935 ; FROM INDIA

THE present report deals with the nematode parasite, *Spironoura brevispiculata* Baylis, 1935 ; obtained from the intestine of five specimens of *Rana hexadactyla* at village Laxmipolavarum, Andhra Pradesh.

*Spironoura brevispiculata* was described by Baylis in 1935 from the intestine of frog, *Rana hexadactyla* at Colombo, Ceylon. A perusal of literature reveals that this worm is not reported so far from India. Hence the author takes an opportunity to put on record for the first time the occurrence of *Spironoura brevispiculata* from the same host in India.

The worms are small in size with a distinct neck. The head is wider. The mouth is surrounded by three lips. Each lip bears two papillae. An inverted Y-shaped cuticular band is present in the buccal cavity. Its main stem has longitudinal striations. There are six inner labial papillae and three cephalic papillae. There are six interlabia separated from three lips by three cuticular band-bearing papillae. The buccal capsule is chitinous. The oesophagus is divided into three parts, viz., anterior tubular, median pseudobulbular and posterior true bulbular portion. The oesophagus leads into the intestine, the connection being internally guarded by three oesophageo-intestinal valves. The posterior end of both the sexes terminates in a tail spine. The cuticular striations are fine.

In his description of *Spironoura brevispiculata* Baylis (1935) remarks "This form differs only in

very minor points from *Spironoura falcata* and examination of further material may possibly show that the differences are not specific". With the plenty of material available, the author had an opportunity to study *Spironoura brevispiculata* and check up its characters.

It is found that *Spironoura brevispiculata* differs from *Spironoura falcata* Vonlinstow (1906) in the following points :

| Nos. | <i>Spironoura brevispiculata</i>        | <i>Spironoura falcata</i>         |
|------|---|-----------------------------------|
| 1    | Submedian papillae absent               | Submedian papillae present        |
| 2    | Inner labial papillae separated         | Inner labial papillae bifurcated  |
| 3    | Six interlabia present                  | Interlabia absent                 |
| 4    | Caudal papillae absent in female        | Caudal papillae present in female |
| 5    | Musculature beyond the cloaca in female | No musculature                    |
| 6    | Presence of tail spine                  | Absence of tail spine             |

The author holds the view that the differences mentioned above are of specific nature and therefore sufficient to recognise *Spironoura brevispiculata* distinct from *Spironoura falcata*.

The author is grateful to Principal, Shri. B. N. Waradpande, and Head of Zoology Department, Dr. R. B. Phansalkar, for providing the research facilities in the department and also to Dr. B. M. Murhar, for taking keen interest in this work.

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#### A CATCH OF THE RARE SNAKE MACKEREL *PROMETHICHTHYS PROMETHEUS* (CUVIER) (PISCES:GEMPYLIDAE) IN INDIAN SEAS

DURING a cruise on the fishing trawler *Tuna* of the Integrated Fisheries Project, Cochin, a specimen of *Promethichthys prometheus* (Cuvier), belonging to the family Gempylidae, was collected off Quilon (Kerala State) by the otter trawl operated at 300 metres. The records of *P. prometheus* are scattered over deep water in the tropical Atlantic and Pacific Oceans (Herre<sup>1</sup>). Except for a solitary report from the East Indies by Bleeker<sup>2</sup>, it has not been recorded so far from the Indian Ocean.

Day<sup>3</sup> in the *Fauna of British India* did not describe any member of the family Gempylidae from the Indian Seas. Alcock<sup>4</sup> recorded the first gempylid, *Thyrstites bengalensis* [= *Rexea prometheoides* (Bleeker)] from Indian waters. Subsequent additions to the marine ichthyofauna were: *Gempylus serpens* Cuvier by Jones<sup>5</sup>, *Epinnula orientalis* Gilchrist and von Bonde by Tholasingam, Venkataraman and Kartha<sup>6</sup>, and *Ruvettus pretiosus* Cocco and *Thyrstoides marleyi* Fowler by Silas<sup>7</sup>. Opportunity is taken here to present a key to the identification of the gempylids known from Indian waters, to facilitate further work on this interesting group of fishes.

#### *Promethichthys prometheus* Cuvier

*Gempylus prometheus* Cuvier<sup>8</sup>, 1831, 213, pl. 222, (type loc. : St. Helena, South Atlantic).

*Thyrstites prometheus*, Bleeker<sup>2</sup>, 1856, 43.

*Promethichthys prometheus*, Jordan and Evermann<sup>9</sup>, 1905, 178, pl. 29.

*Rexea prometheoides* Tholasingam, Venkataraman and Kartha<sup>6</sup> (nec. Bleeker) (partim), 1964, 280.

#### Material

A specimen, 253 mm in standard length, off Quilon (Kerala State) (Lat. 8° 45' N, Long. 75° 50' E), 300 m depth, 12th March 1975, coll. P. K. Talwar ; ZSI Regd. No. F. 7177/2.

#### Description

D XVIII II 16 + 2 ; A III 13 + 2 ; P ii 12 ; V I.

Body moderately elongate and compressed. Depth of body 16.6, length of head 33.6 ; both in percentage of standard length. Diameter of eye 20.6, length of maxilla 41.2, length of snout 35.3, interorbital width 13.6, length of lower jaw 56.4, and length of pectoral fin 45.8 ; all in percentage of length of head. Head compressed, the interorbital space with a rather broad groove pointed posteriorly. Mouth large, somewhat oblique, lower jaw projects, maxillary extends to vertical from anterior third eye diameter. Lateral line single, continuous, with an oblique curve downwards slightly behind base of 4th dorsal spine.

Gill rakers on first arch rudimentary as a series of low spiny elevations on the arch, obsolete on anterior half of arch.

Teeth uniserial in each jaw, minute, conic, 13 in each side of upper jaw and 10 on each side in the lower jaw ; the anterior pair of the lower jaw are strong canines which remain outside when the mouth is closed, six strong canines (three fallen out) in the upper jaw ; a single series of villiform teeth on the palatines, a few villiform teeth on tongue.

Two contiguous dorsal fins, origin slightly behind hind border of preopercle ; anterior and posterior

Key to the Indian *Gempylids*

|  |   |
|--|---|
| 1. 9 to 21 dorsal spines; body oblong or moderately elongate .. 3  |   |
| 2. 30 to 32 dorsal spines; body very elongate .. .. .  | <i>Gempylus serpens</i> Cuvier                  |
| 3. Lateral line single .. .. .   | 5   |
| 4. Lateral line bifurcated at its origin .. .. .   | 7   |
| 5. Pelvic fins well developed (at least in the adults), each with a spine and 5 soft-rays .. .. .  | <i>Ruvettus pretiosus</i> Cocco                 |
| 6. Pelvic fins reduced to a single spine .. .. .   | <i>Promethichthys prometheus</i> (Cuvier)       |
| 7. Body more or less elongate; lower branch of the lateral line running along the middle of the body; pelvic fins, if present, inserted a little behind the bases of the pectorals .. .. . | 9   |
| 8. Body more or less fusiform; lower branch of the lateral line running along the ventral edge of the body; pelvic fins inserted well behind the bases of the pectorals .. .. .            | <i>Epinula orientalis</i> Gilchrist & von Bonde |
| 9. 2 dorsal finlets .. .. .  | <i>Rexea prometheoides</i> (Bleeker)            |
| 10. 5-6 dorsal finlets .. .. .   | <i>Thyrsoideus marleyi</i> Fowler               |

spines of the fin somewhat shorter than the median ones; two detached finlets behind dorsal and anal fin each. Origin of anal fin slightly behind that of second dorsal, the spines minute, stout, first spine separated from the rest of the fin. Pelvic fins are represented by a pair of minute spines in front of the base of the pectoral fin. Caudal fin forked, the lobes nearly equal.

Scales very small, cycloid, deciduous, on body and head including the upper part of snout, maxillary and opercles.

Colour in alcohol, uniformly blackish brown with somewhat blackish cloudings; verticle fins more or less dusky except spinous dorsal which is blackish; pectoral fins dusky in their distal half.

**Remarks.**—The species has already been found in Indian seas but not recognised. Tholasilangam *et al.*<sup>6</sup> described the adult specimens of *Rexea prometheoides* (Bleeker) as 'dark brown' and in which the upper branch of the lateral line absent. These specimens are clearly identical with *Promethichthys prometheus* Cuvier.

The author is grateful to Dr. S. Khara, Deputy Director-in-Charge, Zoological Survey of India, and Shri M. Devidas Menon, Director, Integrated Fisheries Project, Cochin, for their sustained encouragement and facilities.

Zoological Survey of India,  
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# AFTER-DISCHARGE IN THE VENTRAL NERVE CORD OF THE SCORPION *HETEROMETRUS*

THE study of simple photoreceptor systems in animals wherein parts of the nervous system are directly sensitive to light is gaining considerable importance in recent times. Studies were carried on such neural photoreceptors which occur in the metasoma of the scorpion *Heterometrus*<sup>1</sup>. In order to understand the effect of the photic input from these photoreceptors on the functioning of the nervous system, the electrical activity was recorded from units in the ventral nerve cord on photic stimulation of photoreceptors in the metasoma.

Bipolar platinum hook electrodes were used for recording. The activity was displayed on a Tektronix Oscilloscope and photographed using Grass kymograph camera. A tungsten-filament microscope lamp fitted with a heat filter was used for photic stimulation.

The photic stimulation of the metasomatic ganglia and the telsonic nerves was found to elicit spike activity in a large number of units in the ventral nerve cord. These units were silent under complete darkness. It was observed that the activity once elicited by the light stimulus, often lasted for considerable period even after the stimulus was off. The duration of this after-discharge was as long as 20 sec.

One silent unit responded to light stimulation with a latency of about 2.6 sec. and continued to discharge throughout the period of photic stimulation which was about 23 sec. There was an after-discharge which lasted for about 9 sec. after the stimulus was off (Fig. 1). The frequency of

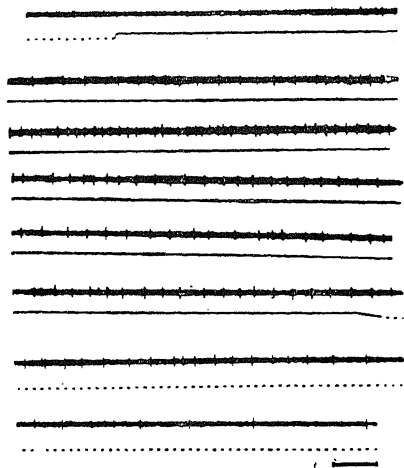


FIG. 1. Sustained activity elicited in a silent unit in the ventral nerve cord on photic stimulation of the seventh abdominal ganglion. The figure represents a single continuous record read from top to bottom. Lower trace: Light stimulus. Time: 0.5 sec.

discharge gradually increased and reached a maximum of 8 to 10 spikes/sec. about 10 sec. after the initiation of the stimulus. The discharge frequency then decreased gradually and was maintained around 3 to 5 spikes/sec. during rest of the period. A prolonged after-discharge has also been reported in the photoreceptor neurons of crayfish<sup>2</sup> and the mollusc *Onchidium*<sup>3</sup>.

The exact mechanism of this after-discharge is not clear. Considering these units to be post-synaptic, the long after-discharge can be said to be due to sustained transmitter action. It may also be due to the presence of presynaptic reverberating circuits or temporal dispersion of presynaptic impulses due to the differences in the conduction velocity or response latency of presynaptic units as suggested by Fielden<sup>4</sup>. Kennedy and Preston<sup>5</sup> have, however, demonstrated after-discharge in units without the presynaptic action.

The electronic equipment used for the study was obtained with the help of Prof. T. H. Bullock, and the late Prof. Kandula Pampapathi Rao, to whom I am indebted, through a research grant from the USAFOSR, to them. I wish to extend my sincere thanks to Dr. A. R. Kasturi Bai, for her kind encouragement.

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#### UTERO-VAGINAL JUNCTION IN THE INDIAN FRUIT BAT, *ROUSETTUS LESCHENAULTI* (DESMAREST)

THE characters of the genital organs and foetal membranes, according to Mossman (1937, 1953), are more reliable for determining the phylogeny and interrelationships of mammals than those of skin, limbs, dentition and alimentary canal since the former, being relatively isolated from direct environmental influence, are more conservative than the latter. During the course of evolution of the mammals there is not only a differentiation of the Mullerian duct in the cranio-caudal axis into three morphologically and physiologically distinct segments—the Fallopian tube, the uterus and the vagina—but also a progressive medianization and union of the two Mullerian ducts from the caudal towards the cranial segment culminating in the formation of a simplex uterus as in primates.

Interestingly, although the bats appear to be a homogeneous group of mammals possessing unique morphological characters adapted for a flying habit, their genital anatomy exhibits wide variations among the different species, thereby raising the question if these animals are monophyletic or polyphyletic.

In *Rousettus leschenaulti* (Megachiroptera) the uterus is bicornuate as in most bats, but while the two cornua are externally united at their caudal regions, their lumina remain separate throughout their lengths and open into the vagina by independent cervical canals (Figs. 1–3). Each of the cervical canals is provided with its own coat of circular muscles which is continuous with the circular muscle layer of the ipsilateral uterine cornu. The cervix projects in the form of an hemispherical bulb into the cranial end of the vagina and is attached to the dorso-medial wall of the vagina being free in the rest of the regions. Hence, in transverse sections the vaginal lumen appears crescentic at its cranial segment.

Jones (1917), while describing the female genitalia of some species of Megachiroptera, mentioned, “the internal genitalia of the female exhibit the curiously primitive condition of completely

separated uteri. In some forms (as *Epomophorus* and *Cynonycteris*) each uterus possesses its own separate of uteri which opens into the common vagina; whilst in others (as *Pteropus*) two elongated uterine cornua are united outwardly in their lower portions into a corpus uteri, but this uterine body

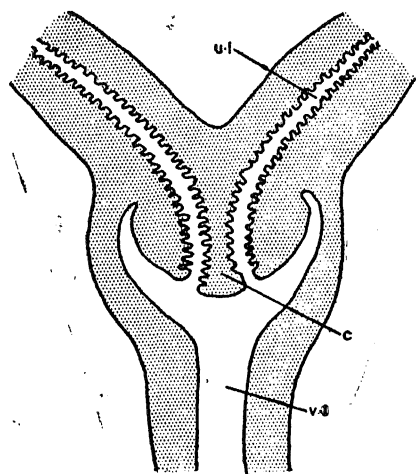
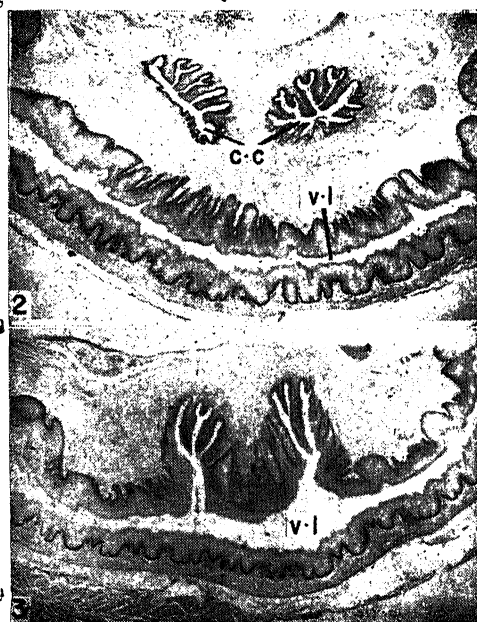


FIG. 1. Semischematic representation of the utero-vaginal junction in *Rousettus leschenaulti*.



FIGS. 2-3. FIG. 2. Photomicrograph of transverse section of a part of the cervix showing the two cervical canals,  $\times 22$ . FIG. 3. Photomicrograph showing the independent opening of the two cervical canals into the vaginal lumen,  $\times 22$ . c, cervix; c. c, cervical canals; u. l, uterine lumen; v. l, vaginal lumen.

is completely septate within". On the basis of his studies of the genital organs he concluded that the Megachiroptera are primitive and form a fairly well defined group. Regarding the Microchiroptera he suggested the possibility of the group being polyphyletic. Ashfaq and Tungare (1960) noted that while in *Cynopterus sphinx gangeticus* the cervix carrying the two independent canals extend to about half the length of the vagina in *Pteropus giganteus giganteus* the vagina has a vertical longitudinal septum, which extends almost its entire length so that the vagina has two independent canals, each of which continues into the lumen of the respective uterine cornu. Thus, in this animal the two sides of the genitalia are completely separated.

In all the Microchiroptera except phyllostomids, the two uterine cornua meet caudally and their lumina become confluent and open into the vagina by a common cervical canal (Jones, 1917; Wimsatt, 1944; Gopalakrishna, 1947). The phyllostomids have a simplex uterus almost resembling that of the higher primates (Hamlett, 1934, 1935; Rasweiler, IV, 1974).

From the foregoing it is evident that the structure of the female genitalia of *Rousettus leschenaulti* is intermediate between the Megachiroptera and the Microchiroptera and can be considered as a 'transitional stage in the change over from a septate vagina (as in the other Megachiroptera) to a common vagina with separate uteri with common cervical canal (as in most Microchiroptera).

I am grateful to Prof. Dr. A. Gopalakrishna, Director, for guidance and encouragement during the progress of this work.

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Nagpur, July 7, 1975.

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## SOME NEW FRUIT ROT DISEASES

DURING June 1974, a survey of market and storage diseases of fruits and vegetables was undertaken. The site selected was Nainital, Bhowali and its adjoining areas. Consequently, four, hitherto, unrecorded diseases of *Capsicum annuum* and *Pyrus communis* var. China were discovered. These were found to be fairly common.

A dry rot of *Capsicum annuum* caused by *Neocosmospora vasinfecta* E. F. Smith was responsible for causing an estimated 5–8% loss in the marketing channels. The disease manifested itself as an ochraceous tawny coloured soft area which appeared dry and was covered with mycelial growth of the organism. When the fruit was cut open, dirty white mycelium was seen covering the seeds adjacent to the necrotic areas. The diameter of the lesions ranged from 1.0 to 1.5 cm. Mature fruits were found to be more susceptible to the disease.

Three soft rot diseases of *Pyrus communis* var. China were observed. *Penicillium purpurogenum* Stoll was found to be causing warm buff coloured soft areas on the skin of the fruit which later changed to zinc orange. The disease advanced in a circular manner and from the soft rot a spore laden juice of the fruit was exuded. A tawny coloured growth of the organism was visible on the diseased spots. About one half of the fruit became infected within a week.

Sayal brown coloured, soft, scattered areas were noticed on *P. communis*. The disease first made its appearance either at the stalk end or through a skin injury on the fruit. The diseased areas emitted a fermented odour. Isolations from the diseased portions invariably yielded *Paecilomyces variotii* Bainier. About one-third of the fruit got destroyed during the course of a week.

Another soft rot of *P. communis* was found to be caused by *Fusarium equiseti* (Corda) Sacc. The disease mostly appeared at the stalk end of the fruit. The lesions were olive buff, circular and gradually increased in diameter involving nearly two-thirds of the fruit.

Percentage of loss due to *Penicillium purpurogenum*, *Paecilomyces variotii* and *Fusarium equiseti* was estimated to be 12–14%, 8–10% and 4–7% respectively.

The respective organisms were isolated from the diseased fruits and their single spore cultures were prepared by the method suggested by Keyworth<sup>1</sup>. The pathogenicity of the respective organisms was established as Koch's postulates were fully satisfied. The pathogens resembled the type species.

Thanks are due to the C.S.I.R. and U.G.C. for financial assistance and to the Director, C.M.I.,

Kew, England, for confirming the identity of the organisms.

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FOUR NOTEWORTHY CORTICOLIOUS  
LICHENS FROM BENGAL

DURING December 1970, a botanical exploration was undertaken in 24-Parganas (West Bengal) and the following lichens were collected; *Graphina perstriatula* (Nyl.) Zahlbr., *Graphina subobtecta* (Nyl.) Zahlbr., *Graphina leucocarpoides* (Nyl.) Zahlbr. and *Phaeographina grisea* (Nyl.) Zahlbr.; these have not been recorded from the mainland of Bengal so far. The specimens mentioned below were identified in the Cryptogamic Laboratory of the Botanical Survey of India, Howrah. The identity was also confirmed by Prof. C. W. Dodge, of the University of Vermont, Burlington, U.S.A. The specimens are deposited in the Cryptogamic Unit, University of Vermont, Burlington, U.S.A., and the herbarium of the Cryptogamic Unit, Botanical Survey of India, Howrah, (CAL).

Graphidaceae

*Graphina perstriatula* (Nyl.) Zahlbr. *Cat. Lich. Univ.* 2 : 418, 1924; *Graphis perstriatula* Nyl. *Bull. Soc. Linn. Normand. Ser. 2*, 7 : 176, 1873. Hue in *Nouv. Archiv. Museum Ser. 3*, 8 : 156, 1891.

Thalli pale greenish-grey, forming smooth crusts upon the bark. Apothecia short, curved, branched, partly immersed. Disk closed; exciple coloured like the disk. Hypothecium hyaline. Spores ellipsoid, 2–4 in each ascus, muriform, measuring  $24\text{--}36 \times 8\text{--}18 \mu$ . (Fig. A).

Similar to *Graphina striatula* but differs from spores, 8 in each ascus, 3–5-septate, oviform.

Geographical Distribution : Andaman.

Specimen Examined : On the bark of *Areca catechu* Linn., West Bengal, 24-Parganas, Bongaon, December, 1970, Roy Chowdhury, 1468.

*Graphina subobtecta* (Nyl.) Zahlbr. *Cat. Lich. Univ.* 2 : 427, 1924. *Graphis subobtecta* Nyl. *Bull. Soc. Linn. Normand. Ser. 2*, 7 : 177, 1873; Hue in *Nouv. Archiv. du Museum Ser. 3*, 3 : 162, 1891.

Thalli whitish, forming crusts on the bark. Apothecia short, curved. Disk closed. Hypothecium hyaline. Spores oblong, hyaline, muriform. 8–9–

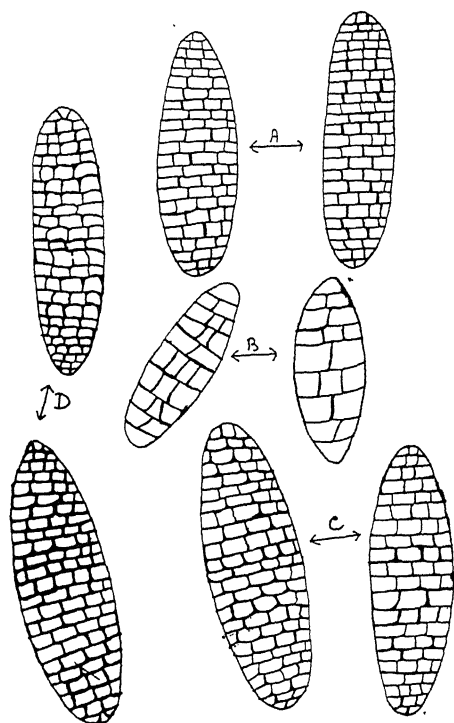
septate transversely and 2-3-septate longitudinally, measuring  $50-110 \times 21-30 \mu$  (Fig. B).

*Geographical Distribution* : Andaman.

*Specimen Examined* : On the bark of *Mangifera indica* Linn., West Bengal : 24-Parganas : Bangaon ; December, 1970, Roy Chowdhury, 1485.

*Graphina leucocarpoides* (Nyl.) Zahlbr. *Cat. Lich. Univ.* 2 : 412, 1924. *Graphis leucocarpoides* Nyl. *Bull. Soc. Linn. Normand. Ser. 2*, 7 : 176, 1873. Hue in *Nouv. Archiv. du Museum Ser. 3*, 3 : 161, 1891.

Thalli whitish, forming smooth crusts on the bark. Apothecia linear, curved, branched. Disk closed. Hypothecium hyaline. Spore single in each ascus, oblong, muriform, measuring  $110-170 \times 30-60 \mu$  (Fig. C).



FIGS. A-D. A. Spores : *Graphina perstriatula* (Nyl.) Zahlbr. ; B. Spores : *Graphina subobtecta* (Nyl.) Zahlbr. ; C. Spores : *Graphina leucocarpoides* (Nyl.) Zahlbr. ; D. Spores : *Phaeographina grisea* (Nyl.) Zahlbr. ;

*Geographical Distribution* : Andaman.

*Specimen Examined* : On the bark of *Cocos nucifera* Linn., West Bengal : 24-Parganas, Bangaon, December, 1970, Roychowdhury, 1470.

*Phaeographina grisea* (Nyl.) Zahlbr. *Cat. Lich. Univ.* 2 : 439, 1924 ; *Graphis grisea* Nyl. Hue in *Nouv. Archiv. du Museum Ser. 3*, 3 : 164, 1891 ; *Acta Soc. Sci. Fennic.* 26 : 22, 1900.

Thalli greyish, forming smooth crusts on the bark. Apothecia linear, curved, branched. Disk open. Hypothecium brownish. Spores brown, oblong-ellipsoid, transversely and longitudinally septate, measuring  $64-108 \times 20-32 \mu$  (Fig. D).

*Geological Distribution* : Ceylon.

*Specimen Examined* : On the bark of *Lagerstroemia indica* Linn., West Bengal : 24-Parganas, Bangaon, December, 1970. Roy Chowdhury, 1562.

I am much indebted to Prof. C.W. Dodge for confirming the identity and Drs. K. Subramanyam and N. C. Nair, of the Botanical Survey of India for encouragement.

Cryptogamic Section, K. N. ROY CHOWDHURY.  
Botanical Survey of India,  
Ind'an Botanic Garden,  
Sibpur, Howrah-3, May 2, 1975.

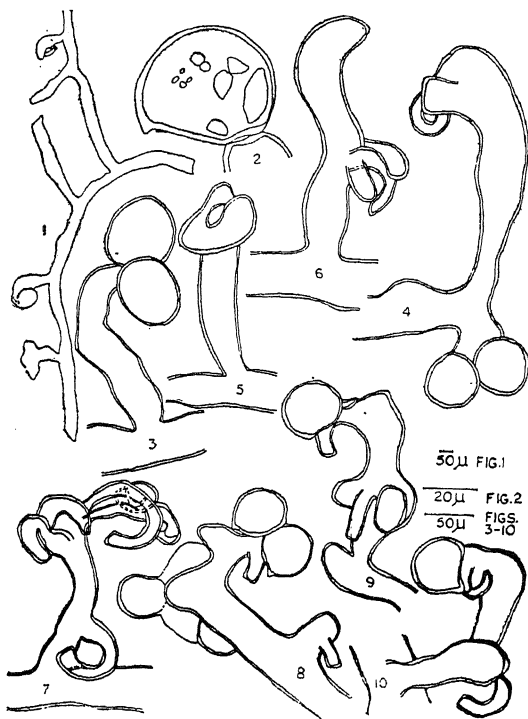
#### ON THE OCCURRENCE OF *VAUCHERIA PSEUDOMONICA* FROM INDIA

*Vaucheria pseudomonica* Fritsch et Rich was for the first time reported from South Africa<sup>1</sup>. Since then its occurrence from other parts of the world is not known. This communication pertains to the occurrence of *V. pseudomonica* from Patiala (Punjab). Dark yellowish green velvety patches of the alga were found growing in the nursery of the Botanical Garden of the Punjabi University. The following observations were recorded from fresh and live material brought to the laboratory during January 1975.

The thallus is terrestrial, branched, monoecious and bears sex organs on special lateral fruiting branches. The filaments are smooth (Fig. 1) and  $24-80 \mu$  broad. The fruiting branches measure  $40-384 \mu$  in length and may either bear both the sex organs or one of them. The oogonia are single (Fig. 2) or in groups of two (Figs. 3 and 4) but not more than two in any case, planoconvex with a distinct hyaline pointed beak on one side (Fig. 2), wall two layered and measure  $44-60 \mu$  broad and  $48-68 \mu$  long. The antheridia are hooked and their position is variable. They are borne either singly (Fig. 5) or in groups (upto five) (Figs. 6 and 7) on special antheridial branches or in between two oogonia (Fig. 8) or below a single oogonium (Figs. 9 and 10). They are  $16-20 \mu$  broad, without a delimiting cell. Dehiscence takes place probably by a terminal pore. The oospores are yellowish green, filling the oogonia, subglobose



(44–60  $\mu$  broad and 52–72  $\mu$  long) to globose (50–60  $\mu$  in diameter), wall two layered: outer dark and thick and inner thin. Zoospores and akinetes are not seen. The present material, however, differs from the type specimen in possessing broader filaments and the absence of a thick, striated wall.



FIGS. 1–10 (For explanation of figures see text).

So far only 9 species of *Vaucheria* are recorded from India<sup>2</sup> of which *V. geminata*, *V. uncinata* and *V. sessilis* are known earlier from Punjab<sup>3</sup>. This forms the first report of *V. pseudomonoica* from Punjab and India.

The author is grateful to Dr. G. S. Venkataraman, for the help rendered during identification and to Prof. S. S. Bir, for facilities and encouragement.

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# EFFECT OF GLUCOSE, SUCROSE AND LACTOSE ON THE GROWTH AND HETEROCYST FORMATION IN A BLUE GREEN ALGA *HAPALOSIPHON WELWITSCHII* W. AND G. S. WEST STRAIN 27

It is evident from the works of Fogg (1949), Wolk (1965), Singh and Srivastava (1968) and Kaushik *et al.* (1971) that cellular differentiation in blue green algae is nutritionally controlled. Heterocysts have been known to fix nitrogen (Stewart *et al.*, 1969) and their formation is governed both by inorganic and organic compounds. Until recently, work on this aspect has been done on some blue green algae and no work is reported on *Hapalosiphon welwitschii* W. and G. S. West. With a view to further understanding the nutritional aspects, the present work has been undertaken.

The alga was raised from a sugarcane field soil, collected at Bailhongal and was cultured in the laboratory. Clonal cultures have been obtained from a single filament from a stock culture, maintained in the algal laboratory of the Botany Department. This was further made bacteria free by repeated subculturing, supplemented with ultraviolet irradiation of the centrifuged material. In order to select the medium suitable for the growth of the alga it was first grown in five different media, all having a pH of 7.5; viz., Molisch's + NO<sub>3</sub> medium<sup>3</sup>, Chu No.-10 + NO<sub>3</sub> medium<sup>3</sup>, Allen and Arnon's + NO<sub>3</sub> medium<sup>7</sup>, Watanabe's - NO<sub>3</sub> medium<sup>9</sup> and Allen and Arnon's - NO<sub>3</sub> medium<sup>6</sup>.

In order to provide a nitrogen free medium, Allen and Arnon's was used as the basal medium in the present study. The algal suspension (10 ml) from the basal medium containing about 5 mg of alga on dry weight basis, was centrifuged, washed thoroughly with distilled water and inoculated in a sterilized medium containing the required amount of the sugar. All the cultures were maintained at room temperature near a north window. Cultures were made in four sets; of these, two sets of 20 day old cultures were filtered, dried and the mean dry weight was recorded (Fig. 2). Other two sets of 20 day old cultures were used to count the heterocysts and recorded as the number of heterocysts per hundred vegetative cells (Fig. 3). Heterocyst frequency thus recorded is the average of 20 observations made for each culture.

Growth of the alga in different culture media is shown in Fig. 1. Among five media tested, Chu No. 10 nitrogen supplemented medium is the most suitable for the growth of the alga. Of the two nitrogen free media tested, the alga showed better growth in Allen and Arnon's medium.

Effect of different concentrations of sugars on the growth of the alga is shown in Fig. 2 and that of

the heterocyst frequency in Fig. 3. From these figures it is evident that in general, lower concentrations of sugars tested accelerate the growth of the alga. Among all the concentrations of glucose supplemented cultures, 0.2% is the most beneficial. Similar is the case with 0.2% lactose, whereas sucrose supplemented cultures showed maximum growth in 0.1%. Among the cultures, those supplemented with 0.2% lactose showed the maximum growth with about three fold increase in the final dry weight. Microscopical observations of the cultures showed that 1.0% lactose is lethal to the alga. In this culture, the filaments were broken, cells separated from each other and many disintegrated. Periodic observations in this, had shown no signs of growth.

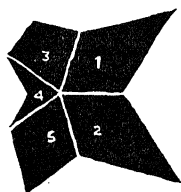
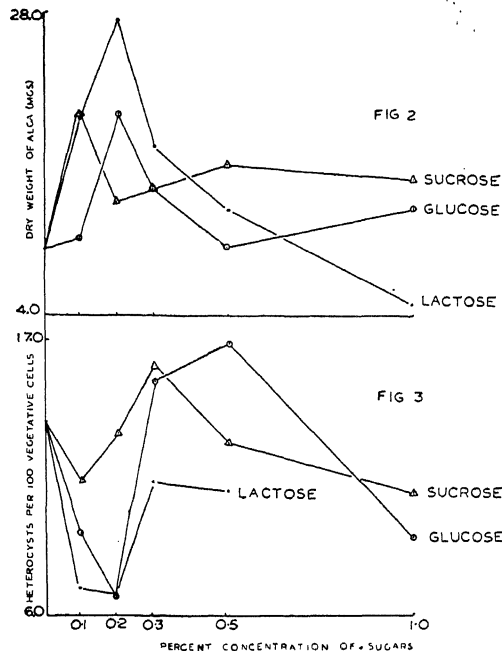


FIG. 1



Figs. 1-3. Fig. 1. Comparative dry weight of 20 day old cultures in 1. Allen and Arnon's +  $\text{NO}_3^-$ ; 2. Chu No. 10 +  $\text{NO}_3^-$ ; 3. Molisch's +  $\text{NO}_3^-$ ; 4. Watanabe's -  $\text{NO}_3^-$  and 5. Allen and Arnon's -  $\text{NO}_3^-$  media. Fig. 2. Growth of alga in sugar supplemented cultures. Fig. 3. Heterocyst frequency in sugar supplemented cultures.

Observations from the heterocyst frequency showed that the maximum number of heterocysts are produced in 0.5% glucose, consequently the total growth was minimum in this and in 1.0% lactose it was lethal. Minimum number of heterocysts are produced in 0.2% lactose, 0.2% glucose and 1.0% sucrose. 0.1% sucrose was also equally harmful. Heterocyst counting was not possible in 1.0% lactose supplemented cultures, due to the disintegration of many cells. In general, concentrations of sugars that favoured the growth of the alga did not favour the differentiation of heterocysts. This is probably due to the continuation of vegetative growth at these concentrations. Watanabe and Yamamoto<sup>9</sup> in their study of heterotrophic growth of *Anabaenopsis circularis*, have suggested the use of favourable concentrations of certain sugars in the mass culture of blue green algae. They reported that certain concentrations of glucose, fructose and sucrose accelerate the growth of the alga. The present work also substantiates their observations.

Lower concentrations of glucose, sucrose and lactose favour the growth of *Hapalosiphon welwitschii* W. and G. S. West., and the same concentrations, however do not favour the formation of heterocysts. The higher concentrations favour heterocyst formation except in lactose where 1.0% proved fatal.

We wish to express our thanks to the University Grants Commission, for the financial assistance to one of us\*. We also express our thanks to Prof. M. S. Chennaveeraiah for the facilities afforded.

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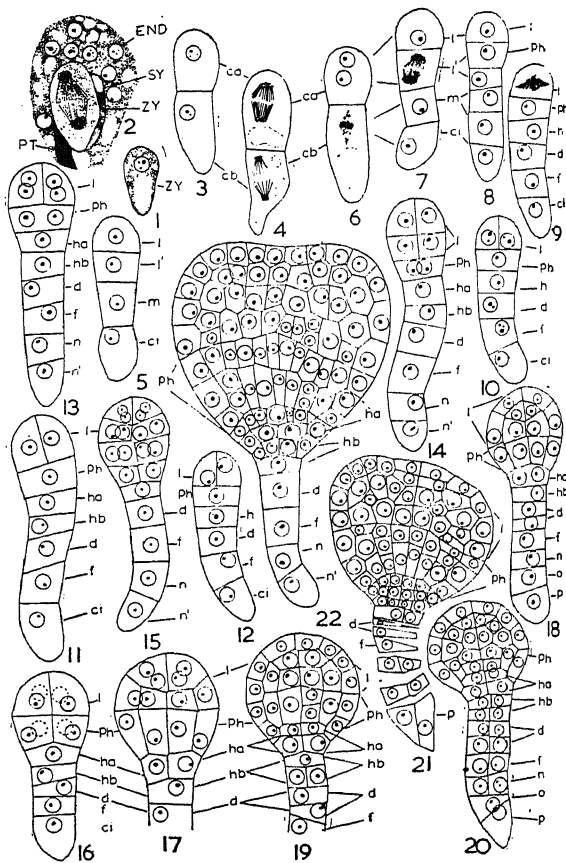
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# EMBRYO DEVELOPMENT IN FIVE SPECIES OF *MOLLUGO*

So far there appears to be no detailed account on the development of embryo in the genus *Mollugo* except that of Bhargava<sup>1</sup> who reported a few stages in the development of embryo in *Mollugo nudicaulis*, but the derivatives and destination of these cells were not described. In view of the lack of definite information, development of embryo has been worked out in detail in five species of *Mollugo* (*M. oppositifolia* L., *M. nudicaulis* L., *M. cerviana* Ser., *M. distichi* Ser. and *M. lotoides* O. Kze.), collected from Andhra Pradesh. Since the sequence of development of embryo among these five species is essentially similar, the development of embryo in *Mollugo* is presented in this communication, giving due attention to the variations, if any, among these species.

The fertilised egg (Fig. 1) after a period of rest enlarges and divides transversely (Fig. 2) to form two superposed cells *ca* and *cb* (Fig. 3). In each of these two cells, transverse wall is laid down simultaneously resulting in a linear tetrad (Figs. 4, 5). However, in *Mollugo cerviana* the transverse division in *ca* is completed earlier than that in *cb* (Fig. 6). Thus at the second cell generation, the four cells of the proembryo are designated as *l*, *l'*, *m* and *ci* (Fig. 5). Subsequently, *l'* and *m* divide by a transverse wall giving rise to *ph*, *h* and *d* and *f* respectively (Figs. 7, 8). When the proembryo is a six-celled filament (Fig. 8), first vertical wall is laid down in the tier *l* (Figs. 9, 10). By about this time, *h* divides transversely resulting in two cells, *ha* and *hb* (Fig. 11) in all species except in *Mollugo distichi* (Fig. 12). This marks the end of third cell generation and the proembryo comprises 8 cells disposed in seven tiers (Fig. 11). Second vertical division takes place at right angles to the first in *l* resulting in a quadrant (Fig. 13). Vertical division in the tier *ph* forms two juxtaposed cells, and *ci* divides transversely forming *n* and *n'* (Figs. 13, 14). Very soon octant formation takes place in the tier *l* (Fig. 15). Each of the two cells of the tier *ph* divides vertically resulting in a tier of 4 cells and *ha* undergoes vertical division to form two cells (Fig. 15). However, in *Mollugo distichi* the divisions in *l'*, *ph* and *ha* are delayed (Fig. 16). Periclinal divisions in *l* and *ph* demarcate dermatogen to the outside (Figs. 17, 18). At about this time the tiers *d* and *n'* divide transversely forming two superposed cells in each of these tiers (Fig. 18). The periblem and plerome initials are laid down in *ph* and the cells of *ha* give rise to semilunar region of cells (Figs. 20, 21) which contribute to the root tip and a part of root cap. The cell *hb* divides at first by a transverse wall and then

by a vertical wall (Figs. 19, 20), of which the upper derivatives form a part of the root cap and the lower ones contribute to the suspensor (Figs. 21, 22). Concomitantly with these changes in *l*, *ph*, *ha* and *hb* vertical wall is laid down either in *d*, *f* and *n* or in *n*, *o* and *p* as in *M. cerviana* (Figs. 20, 21) thus organising a 2-seriate suspensor in this species, while in *M. nudicaulis* and *M. oppositifolia* the suspensor remains exclusively uniseriate (Figs. 18, 22).



FIGS. 1-22. Embryo development in five species of *Mollugo*. Fig. 1. Showing the zygote in division, pollen tube, persistent synergid and free endospERM nuclei,  $\times 500$ . Figs. 2-22. Stages in the development of embryo and for details refer text,  $\times 500$ . (END, endospERM; PT, Pollen tube; SY, synergid; ZY, zygote.)

From the foregoing account it is obvious that the derivatives of the upper four tiers *l*, *ph*, *ha* and *hb*, which are the products of *ca*, contribute to the formation of embryo proper, while the derivatives of *m* and *ci* contribute to the suspensor.

As the terminal cell *ca* of the 2-celled proembryo contributes to the formation of the embryo proper,

the embryogeny conforms to the Solanad type and since hypophysial initial and long suspensor (not haustorial) are present, the embryo development in the five species of *Mollugo* investigated points out to the Linum variation of Solanad type.

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#### ASPIRIN INDUCED CHROMOSOME BREAKAGE IN *ALLIUM CEPA* L.

ACETYL salicylic acid, popularly known as "Aspirin", is well known as an analgesic and is mostly used either as such or in combination with other stimulants in many analgesic and antipyretic drugs. Despite its high therapeutic value, it is not considered as a harmless drug as it was believed earlier since it causes ulcerations in epithelial layers in stomach and chromosome breakage in human leucocytes<sup>1,2</sup>. Mauer *et al.*<sup>3</sup>, reported aspirin to be harmless since it does not significantly increase the chromosome aberrations in human leucocytes at any concentration over the control, while later investigators<sup>4-6</sup>, described them and concluded it to be mutagenic. However, more investigations on its possible cytogenetic effects are necessary in higher organisms. In the present communication, therefore, the results on the effects of aspirin on the chromosomes of *Allium cepa* are described which provide some additional information.

Actively growing root tips of *Allium cepa* were treated with aqueous solutions of aspirin in four concentrations of 0.01%, 0.02%, 0.05% and 0.10%. The frequency and types of aberrations observed following the analysis of cells at metaphase and anaphase in treated as well as controls are given in Table I.

The results indicated that its most immediate effect was the killing and arrest of further growth of cells. Although in lower concentrations some normal cells could be scored, hardly few cells could be seen with normal meta and anaphases in higher concentrations. Among the aberrations scored following treatment, the most conspicuous were the clumping of chromosomes, stickiness of a few to almost all the chromosomes of the entire complement and the erosion of chromatin masses. These showed that the primary effect of aspirin might be the depolymerisation of the nucleic acid in the chromosome. The presence of minute terminal and intercalary breaks followed by reunion at both chromatid and chromosome levels was also detected.

TABLE I  
Aberration types following aspirin treatment

|                   | Percentage of aberrations<br>in treatment<br>(concentration) |      |      |      |      | Average |
|-------------------|--|------|------|------|------|---------|
|                   | Control  | 0.01 | 0.02 | 0.05 | 0.10 |         |
| METAPHASE         |  |      |      |      |      |         |
| Clumping          | 4.7  | 7.0  | 6.7  | 42.1 | 50.0 | 26.5    |
| Chromatin erosion | 0.0  | 14.0 | 21.9 | 32.8 | 27.6 | 24.0    |
| Stickiness        | 2.4  | 11.0 | 8.5  | 16.5 | 14.0 | 12.5    |
| Isolocus breaks   | 0.0  | 7.1  | 1.9  | 1.2  | 0.4  | 2.8     |
| Chromatid breaks  | 0.0  | 1.3  | 5.6  | 0.8  | 0.0  | 1.9     |
| Exchanges         | 0.0  | 1.3  | 6.7  | 0.0  | 1.2  | 2.3     |
| Gaps              | 0.0  | 1.3  | 3.4  | 0.0  | 0.0  | 1.2     |
| Dots              | 0.0  | 0.0  | 6.7  | 2.8  | 4.5  | 3.5     |
| Normal cells      | 92.9   | 57.0 | 38.3 | 3.8  | 2.3  | 25.3    |
| ANAPHASE          |  |      |      |      |      |         |
| Sticky bridges    | 11.1   | 15.5 | 27.2 | 22.5 | 40.0 | 26.3    |
| Chromatid bridges | 0.0  | 6.3  | 9.1  | 15.0 | 11.0 | 10.4    |
| Laggards          | 7.7  | 19.6 | 9.1  | 5.0  | 14.0 | 11.9    |
| Fragments         | 0.0  | 19.6 | 24.0 | 5.0  | 23.0 | 17.9    |
| Normal cells      | 81.2   | 39.0 | 30.6 | 52.5 | 12.0 | 33.5    |

The occurrence of gaps, exchanges and dots in low frequencies and the presence of acentric fragments and laggards at anaphase further pointed out that in addition to partial depolymerisation of nucleic acid, several small to large breaks might be occurring after aspirin treatments. As compared to the controls aspirin had considerably enhanced the aberration frequencies (Table I). Hence it may be considered as a potential chromosome breaking agent in higher organisms.

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**SOFT ROT OF APPLE CAUSED BY  
*CLATHRIDIMUM CORTICOLA* (FCKL)  
SHOEM AND MULLER**

THE occurrence of the soft rot of apple (*Malus pumila*) in India due to *Clathridium corticola* is being reported for the first time. Surveys conducted in the local fruit market and store houses revealed that the disease is common and serious. It rendered the fruits unfit for consumption. The disease was common on Red delicious variety whereas American and Maharaja varieties were less susceptible. The disease manifests itself at first as a small brownish black lesion which enlarges rapidly in diameter and depth. The affected area becomes soft and mushy (Fig. 1).

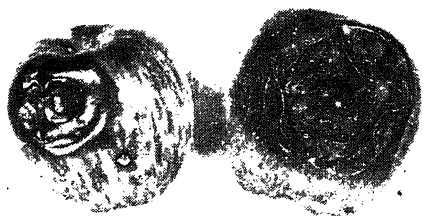


FIG. 1. Diseased fruits of Apple infected with *Clathridium corticola*.

The fungus isolated from the diseased fruits proved pathogenic on inoculations through injured portion of the healthy fruit. Reisolation yielded the same fungus inoculated with. It seems a wound pathogen. In morphology, the fungus resembles with the type description except few variations in the colour of fungal colony and size of the spores. No perfect stage has been observed. Isolate grew well on PDA. It was also grown on Czapek's-malt extract- oat meal- host pulp- and peptone maltose agars where the growth was not luxuriant as on PDA.

The isolate was incubated at an optimum temperature of 28°C. Sporulation was noted when the dishes were kept at low temperatures. The fungus shows psychrophilic tendencies. It is an ascomycete.

This report also constitutes the first record of the genus from India.

The living culture of the fungus has been deposited at I.A.R.I., New Delhi, and the Commonwealth Mycological Institute, Kew, England, under reference No. 191203.

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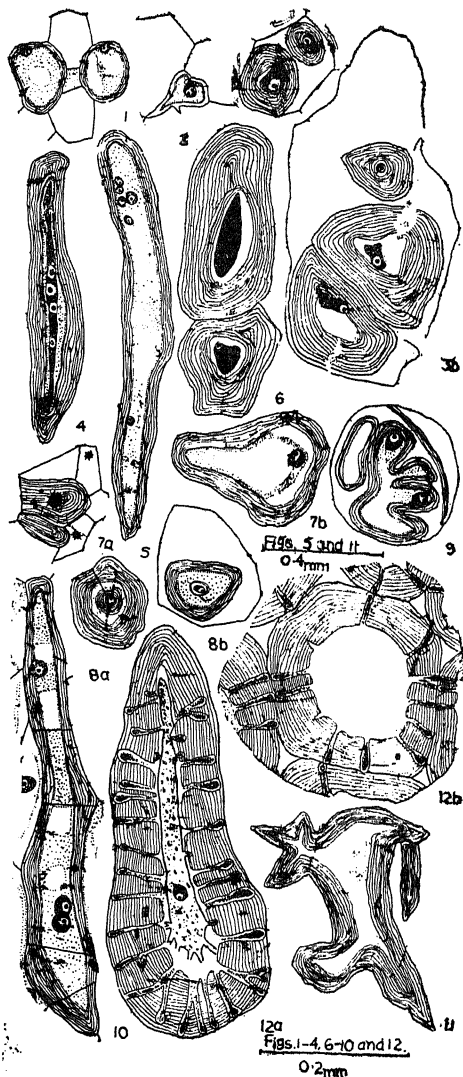
**DEVELOPMENT AND MORPHOLOGY OF  
SCLEREIDS IN SOME SPECIES OF *GNETUM***

SCLEREIDS have been described by Esau<sup>1</sup> as arising "either through a belated sclerosis of apparently ordinary parenchyma cells (secondary sclerosis)" or directly from cells that are early individualized as sclereid primordia. It has been suggested that sclereids make up for the deficiency of lignified tissue in the plant<sup>1</sup>. They have been reported in vegetative as well as floral organs of several higher plants including *Gnetum*<sup>2-5</sup>. The present note deals with some interesting features in their development and morphology of ten species of *Gnetum*, viz., *G. africanum* Welw., *G. columbianum* L., *G. cuspidatum* Bl., *G. gnemon* L., *G. microcarpum* Bl., *G. nodiflorum* Brongn., *G. philippiensis* Warb., *G. scandens* Roxb., *G. ula* Brongn., and *G. venosum* spruce.

The material was prepared for microtomy following standard procedures. Herbarium material was softened in hydrofluoric acid or 5% KOH solution. Embedded material was soaked in a mixture of water, glycerine and acetic acid (5 : 5 : 1) for two weeks prior to sectioning. Sections were cut 8–12  $\mu$  thick and stained with safranin, fast-green or crystal-violet, erythrosin combinations. Material and slides of several species of *Gnetum* were kindly placed at the author's disposal by Professor V. Puri, of the Meerut University.

Sclereids are widely distributed in the plant body in cortex, pith, bract, perianth of male flowers and ovules. A sclereid develops from an ordinary parenchyma cell with dense granular cytoplasmic contents. Such a cell enlarges and the lignin is deposited on the primary wall and soon, secondary walls are also laid down inside, leaving a narrow lumen (Fig. 1). In the development of smaller sclereids, the cells do not enlarge (Fig. 2). During the development of a giant sclereid an ordinary parenchyma cell elongates considerably. The thickenings are deposited in concentric layers and the stratifications marking successive layers of

material deposition are clearly visible. In some cases, giant sclereids seem to be formed from more than one cell. In a few cases, two or three sclereids have been seen developing within a cell (Figs. 3 a, 3 b). Sometimes the nucleus of a developing sclereid increases enormously and occupies the entire lumen of a cell (Fig. 4). Occasionally, a sclereid has, up to six nuclei in its lumen (Fig. 5).



FIGS. 1-12

At maturity sclereids are empty but occasionally, a lightly stained granular substance is present. In *G. nodiflorum* lumen of some the sclereids is occupied by tannin (Fig. 6). Crystals of various

forms are observed in secondary walls, in lumen, nuclei, and nucleoli of sclereids of *G. gnemon* and *G. venosum* (Figs. 7 a, 7 b).

Rarely, a part of sclereid remains unsclerosed and the protoplasm migrates in it (Figs. 8 a, 8 b). "This may be due to the fact that after sclereids have ceased to grow, their apices continue growing, even after full sclerosis"<sup>6</sup>. In many species, secondary walls of sclereids get folded or separated from primary walls (Fig. 9). In *G. scandens*, a few giant sclereids show septa (Fig. 10). According to Rao<sup>7</sup> "the presence of septa constitute an important structural aspect in morphology of sclereids". Sometimes sclereids send out branches in intercellular spaces. Branching may be of various degrees (Fig. 11).

Secondary wall of sclereids, in many cases, is traversed by numerous branched or unbranched pit canals filled with granular content. Pit aperture is either circular, elongated or slit-like (Fig. 12 a). Pit canals of adjacent sclereids lie opposite each other and appear to be connected at times. The chalazal portion of the seed of *G. microcarpum* is completely occupied by such sclereids (Fig. 12 b).

One interesting feature common to all the ten species of *Gnetum* is the presence of macrosclereids (giant sclereids) in the mid-cortical region of the ovule-bearing node. These sclereids give the appearance of tracheids.

The sclereids have been classified into four types, viz., macrosclereids, brachysclereids, astero-sclereids and osteosclereids<sup>8,9</sup>. However, Rao<sup>10</sup>, on the basis of morphological data recognises six main types, i.e., spheroidalsclereids, osteosclereids; fusiform sclereids, filiform sclereids, asterosclereids and crystalliferous sclereids. All these types are present in *Gnetum*. In their structure and development, they show a remarkable similarity with those of angiosperms.

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### COMPLEX INFECTION OF TOMATO (*LYCOPERSICON ESCULENTUM*) WITH CUCUMBER MOSAIC AND TOMATO LEAF CURL VIRUSES

FERN leaf symptoms in tomato are known to be caused by either cucumber mosaic or tobacco mosaic viruses<sup>1</sup> while leaf curl symptom is reported to be produced by tomato leaf curl virus<sup>2</sup>. Diseased seedlings of tomato plants were observed showing both fern leaf and leaf curl symptoms in the year 1972 in the experimental plot of Virology wing, Lucknow University, Lucknow. Such plants showed an extremely stunted growth and failed to survive.

Transmission tests, host range and physical properties showed the presence of Cucumber Mosaic Virus<sup>3</sup> in infected plants. The virus was transmitted more efficiently by aphids in a stylet borne manner and the symptoms in such plants appeared earlier than in mechanically inoculated ones. Characteristic symptoms including filiformity of the leaves were observed in inoculated plants after 7–8 days of virus inoculation. However, the leaf curling symptoms did not appear in either mechanically inoculated test plants or in plants where transmission was done by aphids (*Myzus persicae* Sulz.). The growth in such plants was reduced in comparison to healthy test plants.

In a second test white flies used in the transmission test were given an acquisition feeding of 24 hours on diseased leaves of originally infected plants and were then transferred on healthy tomato seedling for 48 hours. Symptoms including reduction in size of leaves and curling of leaf lamina appeared 10–12 days after transmission. However, none of the inoculated plants showed filiformity of the leaves characteristic of CMV infection.

To establish the complex infection of tomato plants by CMV and TLCV, four batches of tomato seedlings each having 15 plants were selected. First batch was inoculated with CMV (maintained on *Lagenaria vulgaris*) through *Myzus persicae* Sulz. which were given an acquisition feeding of 2 minutes and an inoculation feeding of 24 hours. The second batch was inoculated by tomato leaf curl virus

(maintained on *L. esculentum*) through white flies given an acquisition feed of 24 hours and an inoculation feeding of 48 hours. In the third batch the first inoculation was done with CMV through *M. persicae* and thereafter a challenge inoculation was made after 5 days with tomato leaf curl virus. The last batch was left as control.

Observations revealed the typical symptoms of fern leaf and leaf curl viruses in first and second batch respectively while in third batch an extreme stunting of the plants was noticed accompanied with both fern leaf and leaf curl symptoms. However, the symptoms of leaf curl dominated over fern leaf syndrome. All the plants in this batch died after 25 days of second inoculation. It is evident that the complex form (CMV + TLCV) of the infection can occur in tomato plants posing a threat to the crop in early stages of plant growth. It is inferred that these two viruses have a synergistic action on the host ultimately leading to its premature death.

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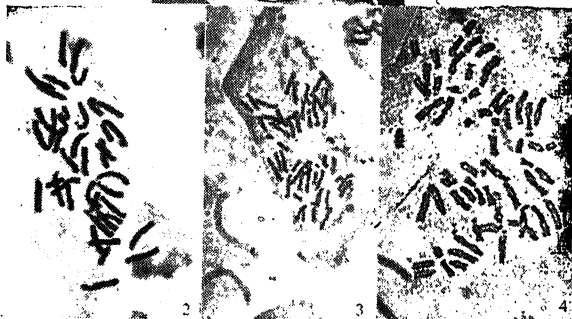
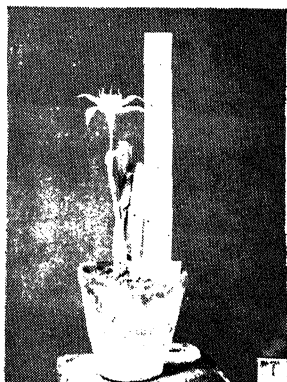
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### VARIATION IN THE SOMATIC CHROMOSOMES OF *PANCRATIUM TRIFLORUM* ROXB.

THE genus *Pancratium* (in Greek, all powerful; referring to its medicinal value) is an Old World genus belonging to the family Amaryllidaceae. This genus includes 14 species whose distribution extends from the Mediterranean eastwards to India and southwards to Africa (Bailey, 1933). *Pancratium illyricum* the "Spider Lily" is the best known in horticulture. Two chromosomal races a diploid  $2n = 22$  (Brumfield, 1941) and a tetraploid  $2n = 44$  (Sato, 1938) are found in this species.

Of the three species of *Pancratium* reported from South India (Gamble, 1915), *Pancratium triflorum* is found in all Districts upto 2,000 ft. It has beautiful scented white flowers with an attractive staminal corona connecting the six stamens (Fig. 1). The bulb of this species is used as a remedy for skin diseases by the tribals of Malabar.

So far there has been no report on the chromosome number of *Pancratium triflorum*. Two clones were collected from Kerala. Squashes from root tips showed that one from Shoranur was a tetraploid  $2n = 44$  (Fig. 2). Two plants collected from a clone in Malapuram District had  $2n = 48$  (Fig. 3) and  $2n = 56$  (Fig. 4) respectively. Further studies on this phenomenon is under way.



FIGS. 1-4. Fig. 1. Photograph of the Plant *Pancratium triflorum* Roxb. Fig. 2. Photomicrograph of somatic metaphase showing  $2n = 44$  chromosomes,  $\times 350$ . Fig. 3. Photomicrograph of somatic metaphase showing  $2n = 48$  chromosomes,  $\times 350$ . Fig. 4. Photomicrograph of somatic metaphase showing  $2n = 56$  chromosomes,  $\times 500$ .

This forms a part of the project "Ethnobotanical Studies of South Indian Aboriginal Tribes" and I wish to thank Dr. E. K. Janaki Ammal, for her guidance.

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## EFFECT OF SALINITY ON SPORE SHEDDING IN *GRACILARIA CORTICATA*

INFORMATION available on factors influencing sporulation and other growth phases on the Indian Seaweeds is meagre<sup>1,2</sup>. Such information being of importance, in the possible cultivation of the economically useful seaweeds of India, some observations were made on the effect of salinity on tetraspore shedding in *Gracilaria corticata* J. Agardh, a common lithophyte occurring in the infralittoral fringe along Visakhapatnam coast<sup>3</sup>. Results obtained from ten experiments involving nine different concentrations of salinity are presented below.

Tetrasporophytes of *G. corticata* collected between 2 and 4 P.M. during spring tides were brought to the laboratory (immersed in seawater). Terminal parts of fertile fronds of about the same age group, with well developed sporangia were cut into small bits; thoroughly washed several times in sterile seawater to free them of all epiphytes. Three or four bits were transferred to petridishes filled with different salinity concentrations ranging from 0‰ to 80‰ for spore liberation. Seawater collected from the inshore area was raised to a salinity concentration of 80‰ by adding common salt and sterilized in an autoclave, to make up the stock solution. Lower grades were made by the addition of requisite quantity of distilled water. Experimental sets of petridishes were kept in a culture chamber at room temperature ( $32 \pm 2^\circ \text{C}$ ) with two 20 watt day light fluorescent lamps, operated on a cycle of 8 hours light and 16 hours dark. The spores liberated into the seawater in the petridishes were counted after 24 hours using a plankton counting chamber and a hand tally counter. From the weight of the material determined at the end of the each experiment in each petridish, the tetraspore output per gram per day was estimated for the different samples. The mean values obtained for the different concentrations of salinity are plotted in Fig. 1.

The tetraspore output varied in different salinities ranging from as low as zero at 0‰ (distilled water) to a maximum of 22,575 spores at 40‰, with noticeable increase from 10‰ upwards. At concentrations higher than 40‰, there was a sudden fall in the spore output, the minimum of zero once again being obtained at 70‰. It can be seen from the respective values plotted in Fig. 1 that greater numbers of spores are shed at salinities between 30‰ and 50‰, which appear to be the optimum range for enhanced tetraspore liberation in *G. corticata*, although the peak values are obtained at 40‰. Salinities lower than 30‰ or those over 50‰ are neither conducive nor positively inhibit

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sporeshedding rates under the given set of temperature and light conditions.

Although the effect of salinity on the growth and distribution of such diverse members like *Chondrus*, *Gigartina*, *Laminaria*, etc., have been studied by previous workers<sup>4,5</sup>, its effect on spore liberation has not been recorded. Matsui<sup>6</sup> who studied two species of *Gloiopeltis*, *G. tenax* and *G. furcata*, observed that tetraspore liberation was not significantly influenced by salinities between 17‰ and 52‰ and that their liberation rates decreased at salinities of 12‰ and 60‰. In contrast, our results show that salinity does effect the spore output, at least in *G. corticata*, and that there is an optimum range at which spore shedding is enhanced. Further studies in this direction on *G. corticata*, *Gracilariopsis*, *Hypnea* and other occurring along the Visakhapatnam coast are in progress.

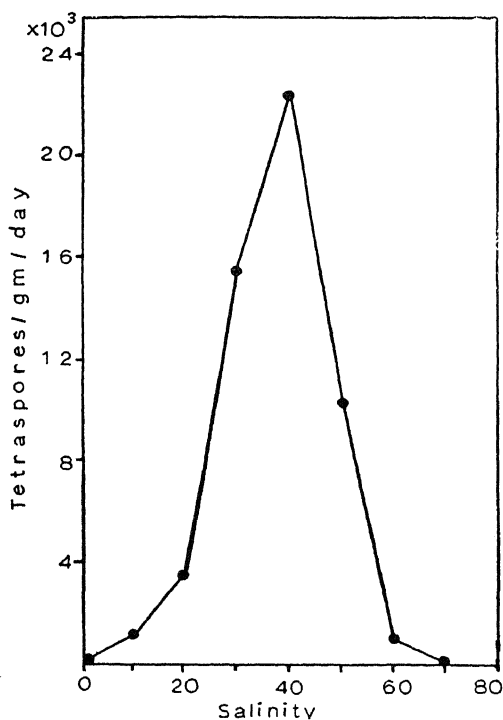


FIG. 1. Influence of salinity on tetraspore output in *Gracilaria corticata*.

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### SPINELESS MUTANT IN *SOLANUM KHASIANUM* CLARKE

COMMERCIAL cultivation of *Solanum khasianum* Clarke is made difficult by its thorny nature. Several attempts to develop spineless mutants have met with varying degrees of success. Notable among these are a curved spine mutant<sup>1</sup> and a less spiny mutant<sup>2</sup>. However, in our mutation experiments, we were able to isolate almost fully spineless forms.

Seeds of *S. khasianum* of a commercial variety having a high alkaloid content (2.0–3.0% on dry weight basis) were given 20 and 30 kr doses of gamma radiation from a Co<sup>60</sup> source. Some of the highly vigorous R<sub>2</sub> plants were treated with 0.01 and 0.02 NMU. A M<sub>3</sub> progeny from the former treatment, produced some plants which are almost completely spineless (Fig. 1). These mutants are

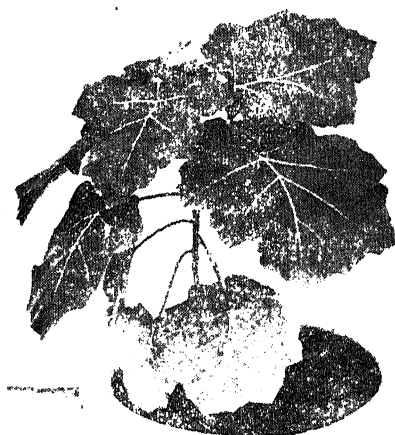


FIG. 1. Spineless mutant in *Solanum khasianum*.

fully fertile and produce fruit profusely. The stems, younger leaves and floral parts are all devoid of thorns. However, an occasional rudimentary spine appears on one or 2 leaves probably due to

differential penetrance or expressivity. Large scale cultivation of the mutant is underway.

The authors are grateful to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, for his interest and encouragement.

Indian Institute of

Horticultural Research,

Bangalore 6, June 23, 1975.

U. R. MURTY.

K. ABRAHAM.

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# A NEW SPECIES OF *THAROOPAMA* SUBRAM.

SUBRAMANIAN (1956)<sup>3</sup> described *Tharoompama* with *T. trina* as the type growing on dead culms of *Cynodon dactylon* Linn. and on dead pods of *Caesalpinia pulcherrima* Sw. In addition to the type species, Subramanian (1956) made *Trichosporium arborescens* Penz. and Sacc. congeneric with *Tharoompama*. *Tharoompama* is a dematiaceous fungus which is characterized by erect, superficial, conspicuous, brownish synnemata with a well defined stalk and fertile head composed of closely aggregated, parallel, septate, brownish hyphae; the fertile head constituted by the individual hyphae which are continued from the stalk, repeatedly branched, becoming progressively free in the fertile part, the ultimate branches (conidiogenous cells) arise in pseudoverticils; the ultimate branches being hyaline to brown, geniculate, cutting off hyaline, globose, one-celled conidia acrogenously. Patil (1964)<sup>2</sup>; Kapoor and Munjal (1966)<sup>1</sup> have reported *Tharoompama trina* Subramanian, growing on fallen leaves of *Ficus hispida* Roxb. and on dead culms of *Cynodon dactylon* Linn., respectively.

During a survey of microfungi from Warangal area, the authors have come across a species of *Tharoompama* Subramanian, growing on dead leaves of *Phoenix sylvestris* Linn. On comparison, the present fungus is found to differ from the other known species in the general morphology, size, shape of the synnemata, conidiophores and conidia, besides occurring on a new substratum. Hence, it is described as a new taxon.

*Tharoompama naimnagarensis* sp. nov. Reddy, Reddy and Manoharachary

Synnemata scattered, conspicuous, superficial, erect with short, simple, dark-brown stalk and sub-hyaline spreading fertile head; synnemata 375–535  $\mu\text{m}$  long; stalk simple, cylindrical, measuring 50–61  $\mu\text{m}$  across at the base and 38.8–50.0  $\mu\text{m}$  in the farther region; the fertile head measuring upto 550  $\mu\text{m}$  in diameter, consisting of individual, loosely arranged, fertile branched hyphae which are con-

tinued from the stalk to form conidiophores; conidiophores sub-hyaline to pale-brown, septate, repeatedly branched (1–3 times), ultimate branches fertile (conidiogenous cells) arranged pseudoverticillately, 25–67.5  $\times$  1.5–2.5  $\mu\text{m}$ , geniculate; conidia produced acrogenously from the tips, conidia hyaline, ellipsoidal and beaked, one-celled, with a basal scar, 3.25–5.6  $\times$  1.3–2.3  $\mu\text{m}$  (Fig. 1).

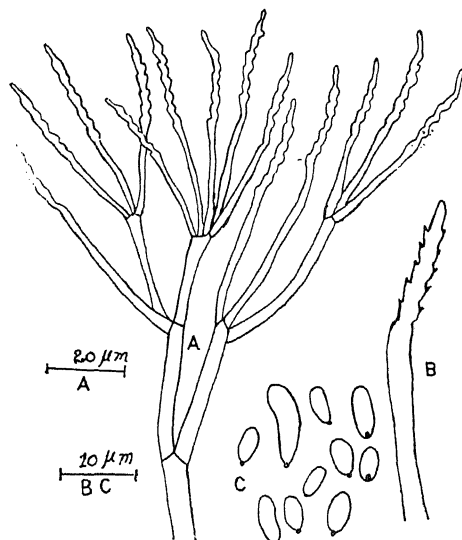


Fig. 1

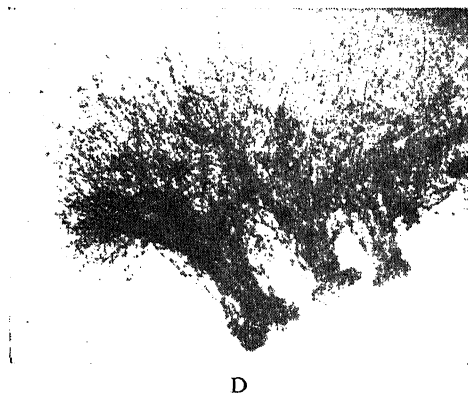


FIG. 1. *Tharoompama naimnagarensis* sp. nov. A, Portion of Conidiophore showing Pseudoverticillate branching; B, Sporogenous cell; C, Conidia; D, Photomicrograph showing the Synnemata,  $\times$  200.

Collected on fallen leaves of *Phoenix sylvestris* Roxb. September 23, 1973; Naimnagar, Warangal, A.P., India. Type deposited in the Department of Botany, Post-Graduate Centre, Warangal, Herb. No. PGCF, 192.

*Tharopama naimnagarensis* sp. nov. Reddy, Reddy and Manoharachary.

Synnemata sparsa, superficialia, erecta, stipitite brevi, simpli fusco-brunneo, capitulo subhyalino, patulo, apicali, synnemata 375–535  $\mu$ m longa, capitulis ad 550  $\mu$ m latis; stipe cylindricus, simplex, 50–61  $\mu$ m crassa ad basim, 38.8–50.0  $\mu$ m latis ad medius, prope basim distentus, *e* hyphis multis longis, pallide brunnei, septatis septis a 45–85  $\mu$ m separatis, compositus, per dimidium apicale fertilis, synnematis hyphis singulis *ex* ordine absolutis *et e* stipite in conidiophora divergentibus; conidiophora pallide brunnea, apicem versus pallescentia, subhyalina extrema hyaline, sub septis ramicantia, ramos secundarios *et* tertiarios pari modo proferentia, tertiariis plerumque pseudoverticellatim ortis, acrogyne sporogenis, rami principalis 2.7  $\mu$ m crassi, secundarii 2.2  $\mu$ m, cellulae sporogenae 25.0–67.5  $\mu$ m longae, 1.5–2.5  $\mu$ m latae; conidia singulatim emissa, sec per incrementum conidiophori sub conidio primo iterum conidia in serie emissa, *et* per repetitionem conidiophora, denique geniculata;

conidia haud septata, ellipsoidea, recta ad basim, hyalina, cicarrici basali notata, 3.25–5.6  $\times$  1.2–2.3  $\mu$ m (Fig. 1).

Thanks are due to Prof. Jafar Nizam, and Prof. U. B. S. Swami, for their kind encouragement and facilities. The authors are also thankful to Dr. P. Rama Rao and Dr. P. Raghuvver Rao, for their helpful suggestions.

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July 31, 1974.

C. MANOHARACHARY.

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## CURRENT SCIENCE

*Fortnightly Journal of Research*

Issued on the 5th and 20th of each month

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Bangalore-6  
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S. R. S. SASTRY  
Manager  
Current Science Association

## SHORT SCIENTIFIC NOTES

### Occurrence of Vitexin in *Jatropha heterophylla*

*Jatropha heterophylla* Heyne (Euphorbiaceae) is a small low glabrous shrub branching from a tuberous root stock with greenish flowers<sup>1</sup>. Jatrophone<sup>2</sup>, a diterpenoid isolated from *J. gossypifolia* and jatropham<sup>3</sup>, a lactone isolated from *J. macrorhiza*, were found to possess antitumour activity. Terpenoids, sterols, flavonoids, alkaloids and carbohydrates were reported from various species of *Jatropha*. We present here the results of our chemical investigation of *J. heterophylla*.

The aerial parts of *J. heterophylla* were powdered and extracted successively with petroleum ether, benzene, chloroform and methanol. Chromatography of the petroleum ether extract residue over silica gel yielded  $\beta$ -sitosterol, besides some long chain alcohols and esters.

The methanolic extract showed positive Shinoda test indicating the presence of flavonoids. It was concentrated under reduced pressure and the aqueous concentrate thus obtained was fractionated into ether, ethyl acetate and *n*-butanol fractions. The ethyl acetate fraction on concentration yielded a yellow flavonoid, m.p. 254–55°. It analysed for the formula  $C_{21}H_{20}O_{10}$  and formed a hepta acetate, m.p. 257–59°. It did not hydrolyse with 2 N HCl but underwent isomerisation. With concentrated HCl hydrolysis took place and the sugar residue was identified as glucose by paper chromatography. Thus it was shown to be a flavonoid C-glucoside. The U.V. spectrum of the glycoside had  $\lambda_{max}$  at 270, 302 (sh) and 335 nm in methanol shifting to 280 and 380 nm with NaOAc, with  $AlCl_3$  to 278, 305 and 386 nm and with NaOEt to 279, 329 and 395 nm. From these properties<sup>4</sup> the compound was identified as vitexin and it was confirmed by Co-PC with an authentic sample of vitexin.

The *n*-butanol fraction showed the presence of vitexin and isovitexin in PC.

The authors are grateful to Dr. V. Krishna Murthy, C.L.R.I., Madras, for authentic sample of vitexin, Dr. V. Venkateswarlu of Osmania University, for supply of plant material and the U.G.C. for financial assistance.

Dept. of Pharmaceutical Sciences,  
Andhra University,  
Waltair 530 003, May 7, 1975.

MRS. N. K. M. LAKSHMI.  
D. VENKATA RAO.  
E. VENKATA RAO.

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### Fossils from Lower Gondwana Formation of Singrimari

C. S. Fox was the first to discover the Gondwana formation in Singrimari (89° 53' 30" E : 25° 38' 35" N) area. On the basis of *Vertebaria indica*, he assigned the formation as Lower Gondwana age in 1935. Four decades after Fox's report, interest on the Lower Gondwana exposure revived, when Gondwana coal was touched in a deep bore hole under the alluvium in Bogra District of Bangladesh. It is presumed that Singrimari exposure is the eastern extension of the Gondwana Group and is separated from its western counterpart by Garo-Rajmahal trough fault<sup>1</sup>.

Recent fieldwork by this directorate, in the area, reveals that the Lower Gondwana exposure is confined to an area of 2 sq. km. Besides *Vertebaria indica* discovered by Fox, fossils recently collected are *Schizoneura* sp., *Gangamopteris* sp., *Glossopteris* sp., *Sphenophyllum* sp. and parts of an invertebrate possibly belonging to Arachnida. Lenticles of coal samples collected from the area analysed as in Table I.

TABLE I

|                                 | Singrimari coal<br>Air dried basis<br>(%) | Average of Lower Gondwana<br>coal of Bihar and Bengal<br>(%) |
|---------------------------------|---|--|
| Moisture                        | 3.3                                       | 1.0  |
| Ash                             | 12.7                                      | 13.0   |
| V.M.                            | 18.5                                      | 22.0   |
| F.C                             | 65.5                                      | 64.7   |
| S                               | 0.7                                       | 0.3  |
| Calorific value<br>6940 KCal/Kg |   | 8,400 KCal/Kg  |

We are grateful to Sri. B. B. Baruah, Officer-in-Charge of Coal Survey Station, Jorhat, Assam, for coal analysis.

Directorate of Geology and Mining, B. C. BAROAH,  
S. K. BHATTACHARYYA,  
Assam, Zoo Road,  
Gauhati-5, April 6, 1975.

1. Chatterjee *et al.*, "Geology and ground water resources of the greater Calcutta Industrial area," *Bull. Geo. Sur. India*, 1964, No. 21, Series B, 1.

### Three Additional Hosts of the Stubby-Root Nematode, *Trichodorus mirzai* Siddiqi, 1960

Moderate to heavy galling was noticed in the roots of *Commelina nudiflora* L., *Eclipta alba* (L.) Hassk. and *Setaria verticillata* (L.) Beauv. growing as weeds in the Aligarh Muslim University Campus. The galls were terminal in position and elongated in shape. The growth of the apical meristem was checked. These are characteristic features for the infection of stubby-root nematodes. The soil around the roots of these plants was isolated and studied for nematodes. It was observed that the soil was heavily infested with *Trichodorus mirzai* Siddiqi, 1960. Whereas the soil from ungalled plants either did not contain this nematode species or, in some cases, negligible numbers were detected. A perusal of literature<sup>1,2</sup> revealed that these plants are new hosts of *T. mirzai*.

Department of Botany, M. MASHKOR ALAM.  
Aligarh Muslim University, S. QAMAR A. NAQVI.  
Aligarh 202001, India, K. MAHMOOD.  
June 7, 1975.

1. Siddiqi, M. R., *Proc. Helm. Soc. Wash.*, 1960, 27, 22.
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### *Cladosporium* Leaf Spot of Sunflower

During October-November 1973, a leaf spot disease of sunflower was noticed around Bangalore. The disease is characterized by greyish circular spots surrounded by a zone of yellow halo. In the initial stages, chlorotic spots appear which, later, turn grey to olive green. The undersurface of the affected part gives a moldy appearance. In severe cases, under high humidity, the spots elongate along the veins resulting in extensive chlorotic patches. The upper half of the leaf usually suffers more damage than the lower half of the leaf.

Isolations from the affected regions consistently yielded a *Cladosporium* sp. When sunflower plants were spray inoculated with spore suspension, from one week old culture of this organism, typical

symptoms appeared after 4 days. The pathogen was reisolated from such artificially inoculated plants.

The pathogen produces an olive-green growth on PDA, which turns dark with the advance age of time. Colony is wooly at earlier stage and becomes powdery later with the production of conidia. Reverse of the colony is greenish-black. Hyaline hypha is not very conspicuous. Dark hyphae measure  $4-11\ \mu$  in width. Conidiophores arise laterally or less often terminally from the hyphae, are unbranched, and measure  $30-420 \times 2.5-5.4\ \mu$ . They are smooth, irregularly septate, not constricted at the septa, and darker and more uniform than the regular hypha. Conidia are produced acropetally in long chains at the tip of the conidiophore and its 3-4 lateral outgrowths. Conidia are smooth, pale brown, mostly 1-celled, ovate or elliptical and slightly tapering at one or both the ends; many cylindrical and often 2-3 celled, mostly towards the bottom of the chain, with one or more hila. Young conidia are almost round in shape. The conidia measure  $2.5-12 \times 2.5-4\ \mu$ .

The pathogen has been identified as *Cladosporium cladosporioides* (Fres.) De Vries<sup>1</sup>. There is no record of this pathogen on sunflower from anywhere. The culture has been deposited in the culture collections of the Department of Plant Pathology, U.A.S., Bangalore, bearing accession No. 102.

Grateful thanks are due to Director, Centraalbureau Voor Schimmelfcultures, Netherlands, for identification of fungus and to Dr. H. C. Govindu, Senior Professor and Head of the Department of Plant Pathology, for facilities.

AICORP Sunflower, T. B. ANILKUMAR,  
Department of Plant Pathology, V. S. SESHADRI,  
University of Agril. Sciences,  
Hebbal, Bangalore 560024, May 1, 1975.

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### Stem Blight of *Asparagus officinalis* Caused by *Phomopsis asparagi* (Sacc.) Bubak

A severe attack of stem blight disease of *Asparagus officinalis* was observed at Hessaraghatta (Bangalore) during the month of January, 1975. It attacked fully grown plants and resulted in their death. The symptoms appeared as a discolouration of the tissues of the stem changing gradually into light brownish areas which became dark brown as the disease advanced. The spots were spindle-shaped initially and were surrounded by deep brown borders. These were more noticeable on the main stems near the ground level but sometimes also appeared on any other part of the stem. In certain

cases, the infection was noticed only on the upper branches, whereas the lower portion of the plant remained healthy. The diseased spots ultimately turned greyish with black dot-like prominent pycnidia. In advanced cases of infection, needles became yellow and the plants were completely or partially defoliated and finally withered.

Pycnidia were scattered, embedded to erumpent, non-ostiolate, opening by a longitudinal slit at maturity, uniloculate but occasionally biloculate and  $90-110 \times 110-220 \mu$ . Conidia were of two types. Alpha conidia hyaline, one celled, oblong to fusoid,  $5.0-10.0 \times 2.5-4.7 \mu$ . Beta conidia hyaline, one celled, filiform, bent,  $12.7-17.5 \times 2.5 \mu$ . These were rarely observed in nature and few in number and were produced in the same pycnidia.

This disease was first described by Kheswalla<sup>1</sup> from Pusa (Bihar) and was reported to be caused by *Phoma asparagi* Sacc. because of the absence of beta spores. These spores have been observed in the present specimen. Hence, the casual organism has been treated as *Phomopsis asparagi* (Sacc.) Bubak<sup>2</sup>.

The authors are extremely grateful to Dr. G. S. Randhawa, Director, for his interest and facilities and Dr. A. Johnston, Director, C.M.I., Kew, Surrey, England, for confirming the identity of the fungus.

|                         |               |
|-------------------------|---------------|
| Indian Institute of     | H. S. SOHI.   |
| Horticultural Research, | B. A. ULLASA. |
| Bangalore, May 9, 1975. | S. S. SOKHI.  |

1. Kheswalla, K. F., *Ind. J. Agric. Sci.*, 1936, 6, 800.
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### Unsaponified Matter of *Asphodelus tenuifolius* Fat

*Asphodelus tenuifolius*<sup>1,2</sup>, known as 'Bokat' in Hindi and belongs to the natural order Liliaceae. Ayurvedic system of medicine described the plant as diuretic and useful for curing ulcers and inflamed parts. In India, it is cultivated widely in Indo-Gangetic plain.

3 kg of dried and powdered plant on extraction with petroleum ether in a Soxhlet extractor and removal of the solvent under reduced pressure yielded 20 g of a brown coloured fat. It was saponified with 0.5 N alcoholic KOH and unsaponifiable matter extracted with solvent ether. The solvent was distilled off and the residue subjected to column chromatography over Brockmann's

alumina using (i) petroleum ether : benzene (2 : 1) and (ii) benzene : chloroform (30 : 25) as eluents. The eluate (i) on concentration and crystallisation from a mixture of chloroform : methanol (1 : 1) yielded white flakes, m.p.  $199^\circ$ ,  $[\alpha]_D^{25} + 87$  (CHCl<sub>3</sub>). Found : C, 84.37; H, 11.86%, m/e = 426; calculated for C<sub>30</sub>H<sub>50</sub>O : C, 84.44; H, 11.81%; acetate, m.p.  $237^\circ$ ,  $[\alpha]_D^{25} + 80$  (CHCl<sub>3</sub>). It gave positive Liebermann-Burchard reaction and Noller's reaction. Mixed m.p. determination and co-chromatography with authentic sample confirmed it as  $\beta$ -amyrin.

The eluate (ii) yielded another colourless compound crystallising from methanol : ethyl acetate (1 : 1), m.p.  $136-7^\circ$ , Found : C, 83.75; H, 12.19%, m/e = 414; calculated for C<sub>29</sub>H<sub>50</sub>O : C, 84.05; H, 12.07%; acetate, m.p.  $127^\circ$ ; benzoate, m.p.  $144^\circ$ . It gave positive Salkowski and Liebermann-Burchard reactions and was identified as  $\beta$ -sitosterol by mixed m.p. and co-chromatography with authentic sample.

|   |               |
|---|---------------|
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| and<br>Department of Chemistry,<br>University of Allahabad,<br>Allahabad (U.P.), India, | R. B. SINGH.  |
| May 22, 1975.   |               |

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### ANNOUNCEMENTS

#### Chemistry of Natural Products

The IV Indo-Soviet Symposium on the 'Chemistry of Natural Products' is scheduled to be held from 18th to 23rd of February 1976 at the Central Drug Research Institute, Lucknow. Topics for the Symposium will also include Biopolymers and Pharmacology of Natural Products.

Abstracts of papers for presentation at the Symposium may be sent by the end of November 1975 to the Project Coordinator, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 001 and may be marked "IV Indo-Soviet Symposium on Chemistry of Natural Products".

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## REVIEWS AND NOTICES OF BOOKS

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**Biomembranes.** (Vol. II). Edited by Lionel A. Manson. (Plenum Press, New York, N.Y. 10011). 1971. Pp. xiii + 302. Price \$ 19.50.

This second volume on biomembranes covers the proceedings of a symposium held at Gatlinburg, Tennessee in 1971. Research activity on the membrane phenomena has exponentially increased in the recent years as evidenced by the number of publications appearing in scientific journals. A spectacular range of problems from chemistry and structure to the pathological aspects of the membranes are currently being investigated. With the rapid growth of literature in this particular line of research activity, there has been an ever increasing need to critically evaluate this material from time to time. The present series publication on membrane associated phenomena is intended to serve this purpose.

The present volume contains 22 articles by specialists on topics of wide ranging interest in the area of biomembrane research besides brief summaries of round table discussions. Current research on structure and chemistry of biomembranes as well as transport mechanisms, control and regulatory functions have been dealt with, like its predecessor, Volume I, the main function of this book could be as a reference volume. The extensive list of literature references appearing at the end of each article serve this purpose well.

Summaries of group discussions have been over-shortened. The usefulness of this book would have considerably increased by including a key word and an author index. As a source of literature information for workers in membrane research, this book would be very valuable.

N. V. S. S.

**Biology of the Land Plants.** Edited by V. Puri, Y. S. Murty, P. K. Gupta and D. Banerji. (Sarita Prakashan, 175, Nauchandi Grounds, Meerut, U.P.), 1974. Pp. xi + 433. Price Rs. 120-00.

This is a collection of papers presented at a symposium on 'Biology of the Land Plants' held at Meerut University from June 18-20, 1972. The symposium was jointly sponsored by the Meerut University and

the University Grants Commission. The book contains fifty research papers dealing with plant morphology and anatomy, developmental morphology and morphogenesis, cytology and cytogenetics, taxonomy, floristics, etc.

The volume reflects the vigorous activity in botanical research in our University. However, it is somewhat disturbing that the research work is still largely descriptive and an experimental approach is only just emerging.

The printing and get-up of the volume is satisfactory.

K. RAMAKRISHNAN.

**Psychopharmacology Communications** (Vol. 1). No. 1, 1975. Edited By Earl Usdin. Publishers : Marcel Dekker, New York, Subscription : \$ 35.00 + \$ 6.30, postage per annum.

This newly introduced journal aims to "provide a means for the rapid publication of well refereed papers in the entire field of psychopharmacology".

The clinical studies presented deal with the pharmacological effects of some centrally active 'phenethylamines', 'Dose-activity relationship of clonazepam', 'possible adverse cardiac interaction with Hydroxyzime hydrochloride' and 'The alterations in the cellular-mediated immune responsiveness of chronic marihuana smokers'.

Biochemistry of the brain is covered by a number of contributions. The reports include 'The effect of hemicholinium on brain acetylcholine and choline in rats, and of 5, 7-dihydroxytryptamine on tryptophan hydroxylase', 'spectral changes in the respiration chain of cerebral cortex slices', 'the stereoscopic binding of morphine to phosphatidyl serine', 'the effects of genetic determination of aggressive behaviour and brain cyclic AMP' and a detailed account of 'a rapid, simple and sensitive fluorescence technique for demonstration of central catecholamine-containing neurons'.

With its aim to achieve rapid publication of papers, the 'communications' can serve as a media for quick information transfer of current research in various laboratories.

M. SIRSI.

# Current Science

Fortnightly Journal of Research, Bangalore, India

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OCTOBER 20, 1975

[No. 20

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# DETERMINATION OF ELECTRON DRIFT VELOCITY IN GAS DISCHARGE PLASMA BY TRANSIT TIME MEASUREMENTS

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**O**BSERVATIONS have been reported on the electron drift velocity in gases based on transit time measurements, in the case of electron swarms<sup>1-3</sup>. The present paper reports the results of similar investigations for a gas discharge plasma where the electron number density is considerably higher  $(6-33) \times 10^{19}$  per  $\text{cm}^3$ . A cylindrical plasma (radius = 1.95 cm) is formed in argon (gas pressure = 0.20–0.60 Torr, discharge current = 1.50–5.00 mA) in a discharge tube, provided with aluminium electrodes. The electrodes are made in the form of a central circular sector and an outer concentric ring, insulated from it; only the central region (radius = 1.0 cm) is used for the actual current density measurements, in order to reduce the error due to the radial decrease in the number density of the electrons due to diffusion and other effects<sup>4,5</sup>. The cathode is provided with a central aperture<sup>6</sup> (radius  $\approx$  0.4 mm) through which are supplied electrons from an auxiliary d.c. maintained gas discharge, in argon, at the same pressure. The electron injection has the effect of making the length of the cathode dark space negligibly small so that the plasma column length approximately equals the interelectrode distance  $D$ , for the main discharge (which can be varied). It also reduces the maintenance potential  $V_{co}$  for the main discharge and generally gives rise to a well-behaved<sup>7</sup> plasma, free from spatial irregularities. The number of electrons injected is time modulated by the superposition of a rectangular voltage pulse (height = 70 V, duration = 150  $\mu\text{s}$ , repetition frequency = 1.6 kHz, rise time  $<$  0.02  $\mu\text{s}$ ) from a pulse generator, on the main voltage of the auxiliary discharge. The increased density of the electrons is followed in its course through the plasma, with the help of two insulation encased circular capacitor probes  $P_1$ ,  $P_2$ , positioned on the outside of the discharge tube; probe  $P_1$  is kept fixed at the cathode, the distance of probe  $P_2$  can be varied. The drift velocity of the electrons is determined by measuring the time delay between the signals picked up by the two probes, with the help of a calibrated double beam C.R.O. (Tektronix type 547). The transit time is measured as a function of the distance between  $P_1$  and  $P_2$  and its average value  $t_d$  for traversing one cm length of the plasma, is determined graphically in order

to eliminate end effects<sup>2</sup> and to ensure that the measurements refer to equilibrium conditions.

It is necessary to verify that the velocity thus determined represents the average drift velocity of the electrons and not the velocity of propagation of a voltage/current perturbation in the plasma. It is known<sup>8</sup> that the velocity with which such a perturbation travels through the plasma is of the same order as the velocity of the acoustic waves, given by

$$\bar{V} = (\gamma k \bar{T}/M)^{1/2} = (\gamma P_0/\rho_0)^{1/2} \quad (1)$$

where  $M$  refers to the mass of the argon. The velocity calculated from this expression comes out to be of the order of  $(5-9) \times 10^3$  cm/sec for our pressure conditions. Donahue and Dieke<sup>9</sup> have reported a velocity for the longitudinal potential waves through the plasma of argon ( $P_0 = 12$  mm) of  $(5.3-7) \times 10^3$  cm/sec; whereas Chiplonkar and Rane<sup>10</sup> have reported a velocity of  $(12-15) \times 10^3$  cm/sec for a self-generated potential wave, in a gas discharge plasma, in hydrogen ( $P_0 = 0.35-0.10$  Torr,  $I_{co} = 10-5 \mu\text{A}$ ). All these values are much smaller than the electron drift velocity observed by us from the transit time data. It is clear, therefore, that for our experimental conditions, the perturbation produced in the electron density does not propagate as a wave.

Another important point which requires consideration is whether under the given experimental conditions, the electron conduction current involves an actual transport of the electrons, from one end of the plasma to the other or involves a collective phenomenon as in the case of conduction by free electrons in a metal wire. In the latter case, the velocity determined from the transit time observations will not have the significance of a drift velocity. The current density  $J_A$  at the anode, can be expressed as  $J_A = N_{eo} q_e V_{dp}$  where  $N_{eo}$  = number density of the electrons in the plasma,  $V_{dp}$  = drift velocity of the electrons near the anode region. Measurement of  $J_A$  (directly) and of  $N_{eo}$  by the Langmuir single probe enables an estimation of  $V_{dp}$  to be made. The axial anode current has been measured by means of a milliammeter, connected between the central sector and the earth. It would be reasonable to conclude that the phenomenon involves an actual transport

of the electrons, if  $V_{dp}$  calculated in this manner could be shown to be smaller than  $V_{dT}$  found directly from the transit time measurement (*vide supra*). This is clearly borne by typical data shown below.

Gas — argon.

$P_0$  = gas pressure (Torr)

$I_{co}$  = discharge current (mA).

$J_A$  = anode current density (A/cm<sup>2</sup>).

$V_{dr}$  = electron drift velocity from transit time (10<sup>6</sup> cm/sec).

$V_{dp}$  = electron drift velocity from probe data (10<sup>6</sup> cm/sec).

| $P_0$ | $I_{co}$ | $J_A$ | $V_{dT}$ | $V_{dp}$ |
|-------|----------|-------|----------|----------|
|       |          |       | ±1%      | ±5%      |
| 0.20  | 1.5      | 4.8   | 1.8      | 0.19     |
| 0.20  | 5.0      | 15.9  | 3.3      | 0.31     |
| 0.30  | 5.0      | 15.9  | 2.3      | 0.29     |
| 0.45  | 5.0      | 15.9  | 1.8      | 0.39     |
| 0.60  | 5.0      | 15.9  | 2.0      | 0.88     |

The longitudinal electric field  $E_0$  in the plasma, has been measured from (i) the observation of  $V_{co}$  the maintenance potential with D, at constant  $I_{co}$  and also from direct measurements with the help of static probes provided in the anode. The values by both these methods, were found to be in agreement with each other. Figure 1 presents typical data obtained in this manner. The reduced field  $E_0/P_0$  has magnitudes between 30.0–33.3 volts/cm Torr for the conditions used here.

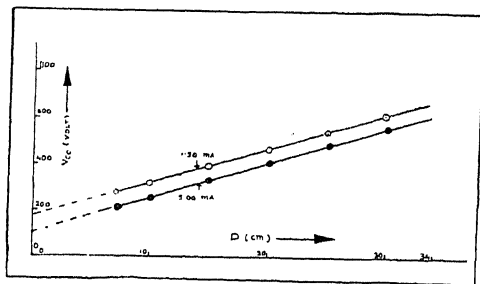


FIG. 1. Variation of maintenance voltage  $V_{co}$  for the total discharge with interelectrode distance D. Gas: Argon.  $P_0 = 0.45$  Torr.

Observations on the electron transit time for different travel distances in the plasma are shown in Fig. 2. It will be noted that the graphs are linear, except near the cathode end. The energetic electrons, injected in the main discharge, appear to require to travel some distance in the plasma, before they attain their equilibrium terminal

drift velocities, as a result of collisions and of the longitudinal electric field. Values of  $V_{dT}$  obtained from these data vary between  $(1.2-3.3) \times 10^6$  cm/sec. They appear to be reasonable in comparison with  $V_d = 1.8 \times 10^6$  cm/sec for  $E_0/P_0 = 4.5$  V/cm Torr given in the literature<sup>11</sup>. The electron drift velocity, observed here, appears to show a small increase with increase in  $I_{co}$ , at constant  $P_0$ .  $E_0$  has been observed to show practically no variation with  $I_{co}$ . The probe data, however, show that under these conditions,  $T_e$ , the electron temperature has a small decrease with increase in  $I_{co}$ .

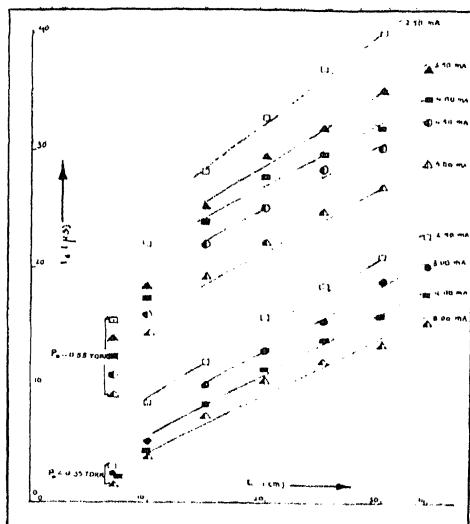


FIG. 2. Variation of electron transit time  $t_d$  with  $L$ , travel distance in plasma. Gas: Argon.

TABLE I

| Gas—Argon | $P_0$ 0.20 Torr |
|-----------|-----------------|
| $I_{co}$  | $T_e$           |
| in mA     | in eV           |
| 1.5       | 3.11            |
| 5.0       | 2.50            |

The change in  $V_{dT}$  with  $I_{co}$  may, therefore, have a real physical significance.

The results show that the mobility description for the motion of the electrons in the plasma is sufficiently accurate for  $E_0/P_0$  as high as 30.0 volt/cm Torr. The electron mobility has magnitudes between  $(0.67-2.44) \times 10^5$  cm<sup>2</sup>/volt sec. The method of observation appears to be fairly accurate and can be used in turn to calculate the number density of the electrons in the plasma under certain conditions.

One of the authors (VVW) is thankful to the Ministry of Education, Government of India, for the research training scholarship.

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## PARTIAL PURIFICATION AND ANTITUMOUR ACTIVITY OF L-ASPARAGINASE FROM *AZOTOBACTER VINELANDII* \*

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### ABSTRACT

Stimulation of L-asparaginase activity in *Azotobacter vinelandii* has been attempted using a variety of carbon and nitrogen sources. Though the tested carbon sources failed to induce the enzyme, nitrogen sources such as ammonium salts, urea, L-aspartic acid and L-glutamic acid were found to be good inducers. The partially purified enzyme preparation possesses antitumour activity against Yoshida ascites sarcoma in rats.

### INTRODUCTION

**L**-ASPARAGINASE from microbial source has been widely used in the chemotherapy of asparagine dependent tumours<sup>1</sup>. Clinical results have shown that L-asparaginase from *Escherichia coli* causes toxicity<sup>2</sup> and immunosuppression<sup>3</sup> in addition to development of resistance<sup>4</sup>. This necessitated studies on L-asparaginases from different sources as a possible chemotherapeutic agent against cancer. In this paper, we report the production, partial purification and antitumour activity of L-asparaginase from *Azotobacter vinelandii*, a nitrogen fixing organism which could be readily cultivated on a large scale in an inexpensive medium.

### MATERIALS AND METHODS

*Azotobacter vinelandii* OP (obtained from Dr. R. H. Burris, University of Wisconsin) was maintained on modified Burk's nitrogen free medium with sucrose as the carbon source<sup>5</sup>. For regular experiments, the organism was routinely cultured in Erlenmeyer flasks, and incubated on a rotary shaker (250 rpm) at 30° C. A 10% inoculum was used throughout the experiments.

Protein was estimated by the method of Lowry *et al.*<sup>6</sup>. Enzyme was assayed essentially as described by Jayaram<sup>7</sup>. One unit is the amount of enzyme required to produce 1  $\mu$  mole of ammonia at 37° C for 30 min. at pH 7.4.

### RESULTS AND DISCUSSION

#### *Studies on the Production of L-asparaginase*

The specific activity of L-asparaginase in different phases of growth of the organism remained almost the same. As the enzyme activity was found to be low (specific activity 2.2) for being exploited for large scale preparative purpose, growth studies were carried out to increase the enzyme yield.

The following carbon sources were tested individually for their ability to stimulate the enzyme activity; glucose, galactose, mannitol, sodium benzoate, lactose, ethanol, and mannose. These carbon sources supported good growth of the organism, but failed to stimulate the synthesis of the enzyme. The nitrogen sources tested were added to the complete growth medium with sucrose as carbon source. Table I summarizes the effects of various nitrogen sources tested for the increased production of the enzyme. Many of the complex nitrogen sources like yeast extract, bacto-tryptone,

\* Presented at the annual meeting of the Association of Microbiologists of India in December 1974.

TABLE I

Effect of nitrogen sources on the induction of  
L-asparaginase

| Nitrogen source (1%) | Specific activity |
|----------------------|-------------------|
| Yeast extract        | 1.97              |
| Bacto-tryptone       | 2.08              |
| Proteose peptone     | 2.10              |
| Meat extract         | 2.08              |
| Casein hydrolysate   | 2.15              |
| L-Asparagine         | 2.53              |
| L-Glutamine          | 2.40              |
| L-Aspartic acid      | 3.75              |
| L-Glutamic acid      | 3.58              |
| DL-Aspartic acid     | 3.70              |
| L-Histidine          | 3.20              |
| L-Arginine           | 3.15              |
| L-Lysine             | 3.30              |
| Potassium nitrate    | 2.0               |
| Sodium nitrate       | 2.3               |
| Ammonium nitrate     | 2.63              |
| Ammonium sulphate    | 3.60              |
| Ammonium phosphate   | 3.16              |
| Ammonium chloride    | 3.70              |
| Ammonium oxalate     | Growth inhibited  |
| Urea                 | 3.70              |

proteose peptone, casein hydrolysate and meat extract were capable of influencing growth of the organism with the increased yield of the biomass, but none of them effected increase in the specific activity of the enzyme. In the case of *Erwinia aroideae* addition of yeast extract to the growth medium has been reported<sup>8</sup> to increase the specific activity of L-asparaginase in crude extracts. However, in *Azotobacter vinelandii*, most of the amino acids tested significantly enhanced the activity. Whereas L-aspartic and L-glutamic acids induced<sup>†</sup> the enzyme, their corresponding amides L-asparagine and L-glutamine had little effect. This kind of induction by the product L-aspartic acid has been observed in *E. coli*<sup>9</sup> and *Pseudomonas* sp.<sup>10</sup>

Of the inorganic nitrogen sources tested, potassium nitrate, sodium nitrate and ammonium nitrate supported good growth, but did not show any stimulation. However, ammonium chloride, ammonium phosphate and ammonium sulphate

induced the enzyme to a similar extent as L-aspartic and L-glutamic acids. Failure of ammonium nitrate to induce the enzyme is most likely due to the preferential utilization of nitrate N than the ammonium N. In *Azotobacter* species Aso *et al.*<sup>11</sup> have shown that the nitrite and possibly nitrate nitrogen first disappeared than the ammonium nitrogen in the growth medium. The enhanced enzyme formation caused by urea is due to its *in vivo* conversion to ammonium ions. From these studies it is clear that the products of the enzyme reaction, *viz.*, L-aspartic acid and ammonia induce fresh enzyme synthesis rather than the substrate L-asparagine.

Table II depicts the induction of L-asparaginase by varying concentrations of ammonium sulphate, L-aspartic and L-glutamic acids. Maximal stimulation (80–90% increase in specific activity) is obtained at an inducer concentration of 1.4%. At lower concentrations ammonium sulphate was found to be more effective than L-aspartic and L-glutamic acids. Increase in the inducer concentration above 1.4% resulted in a gradual decrease in the specific activity. Two per cent and above inhibited the growth of the organism.

TABLE II

Optimum concentrations of inducers for  
L-asparaginase production

| Amount of Inducer (%) | Specific activity |                 |                 |
|-----------------------|-------------------|-----------------|-----------------|
|                       | Ammonium sulphate | L-Aspartic acid | L-Glutamic acid |
| 0.6                   | 3.25              | 2.5             | 2.7             |
| 0.8                   | 3.26              | 2.8             | 2.9             |
| 1.0                   | 3.60              | 3.7             | 3.58            |
| 1.2                   | 3.78              | 3.45            | 3.8             |
| 1.4                   | 4.10              | 4.06            | 3.85            |
| 1.6                   | 3.84              | 3.82            | 3.74            |
| 2.0                   | 3.46              | 3.60            | 3.10            |

#### Purification of the enzyme

Cell-free extracts were obtained by grinding the cells (20 g wet weight) along with glass powder using 200 ml of 0.01 M potassium phosphate buffer (pH 7.4) for extraction. The cell debris was removed by centrifugation of the extract at 27,000 g for 20 min. All the operations were carried out at 0–4° C. The crude extract with a specific activity of 4.0 was treated with protamine sulphate (1 mg for 10 mg protein) to remove the nucleic acids. The supernatant obtained was subjected to ammonium sulphate fractionation. The fraction

† The term 'induced' has been used to indicate fresh protein synthesis as shown by further experiments using inhibitors (unpublished data).

TABLE III

Anti-YAS activity of L-asparaginase in rats

| Dose*                          | Route | No. of injections | % of survivors† | Survivors No. of days of each rat | Average‡ survival period |
|--------------------------------|-------|-------------------|-----------------|-----------------------------------|--------------------------|
| Control                        | i.p.  | 1                 | ..              | 7/3/6/3/5/3                       | 4·5                      |
| Single dose 1000               | i.p.  | 1                 | 83              | 33/4/60/60/9/7                    | 29·8                     |
| „ 2000                         | i.p.  | 1                 | 83              | 19/60/15/60/19/4                  | 29·5                     |
| „ 3000                         | i.p.  | 1                 | 66              | 5/7/60/5/7/60                     | 26                       |
| „ 4000                         | i.p.  | 1                 | 83              | 5/7/5/5/60/7                      | 14·8                     |
| Intermittent doses 800/800/400 | i.p.  | 3                 | All surviving ‡ | 5/5                               |                          |

\* Units/kg body weight given 24 hours after transplantation. Controls injected with 0·005M, 7·4 pH phosphate buffer.

† Survivors at the time of death of the controls.

‡ Experiment was terminated after 60 days.

§ Surviving upto 150 days.

obtained between 30–60% was dissolved in a minimal amount of 0·05 M Tris–HCl buffer, pH 8·4 and chromatographed on a Sephadex G-150 column (void volume 135 ml) with 0·05 M Tris–HCl buffer, pH 8·4, containing 0·1 M KCl as the eluant. Fractions containing enzyme activity were pooled (195–235 ml), concentrated and used in the investigation of antitumour activity. This enzyme preparation was 5 fold pure with a specific activity of 20.

#### Antitumour activity

The Yoshida ascites sarcoma<sup>12</sup> (YAS) was maintained in the substrain of isogenic wistar rats (A/lsc.). Approximately  $3 \times 10^7$  YAS cells taken from the peritoneal cavity of rats bearing a 5 day old tumor were injected intraperitoneally into the experimental rats weighing 100–110 g. Two y-four hours after the tumour transplantation, L-asparaginase was administered intraperitoneally in a single dose and in three intermittent doses with an interval of 24 hrs between each administration. Control animals received same volume of phosphate buffer. The survival rate of the treated animals was scored against the control.

As evident from Table III, administration of 2,000 units/kg body weight, of L-asparaginase provided maximum protection against the tumour. It can also be seen that administration of increasing units of the enzyme results in a decrease in the survival period. This could be due to the toxicity of the enzyme preparation at high concentrations. It is significant that 2,000 units/kg given in 3 split doses protected all the rats.

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# A PALYNOLOGICAL APPROACH TO THE STUDY OF QUILON BEDS OF KERALA STATE IN SOUTH INDIA

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## ABSTRACT

The paper deals with the main results of a palynological study of the Quilon beds exposed at Padappakkara, Paravur and Edvai in the Kerala State of South India. A large number of hystrichosphaerids, acritarchs, numerous kinds of spores of pteridophytes and pollen grains of angiosperms and a fairly good number of fungal fruit bodies and spores have been recovered from the clays of these beds. The pteridophytes are represented by Lycopodiaceae, Gleicheniaceae, Ophioglossaceae, Schizaceae, Adiantaceae, Dicksoniaceae, and Polypodiaceae, of which the spores of Polypodiaceae-Dicksoniaceae complex constitute the predominant elements. The angiosperms are represented by the pollen of Potamogetonaceae, Aroideae, Palmae, Liliaceae, Lemnaceae, Gramineae, Nymphaeaceae, Menispermaceae, Euphorbiaceae, Vitaceae, Combretaceae, Ebenaceae, Lorantheae, Dipterocarpaceae, Sapindaceae, Oleaceae, Lecythidaceae, Simarubaceae, Rubiaceae, Araliaceae, Symplocaceae, Anacardiaceae, Rhizophoraceae, Bombacaceae, Hippocrateaceae, Caesalpiniaceae, Aquifoliaceae, Nyssaceae, Caprifoliaceae, Compositae, Ctenolophonaceae, Labiateae, Meliaceae, Sapotaceae, Polygalaceae, Proteaceae, Haloragaceae, Sonneratiaceae, Olacaceae, Thymeliaceae, Moraceae and Droseraceae.

The abundance of Polypodiaceae-Dicksoniaceae spores, and the occurrence of *Pteridacidites*, *Intrabaculisporis*, *Eximiospora*, *Cingulatisporites*, *Cibotidites* and the pollen grains referable to Compositae (*Compositipollenites*), Sonneratiaceae (*Verrutrisporites*), Dipterocarpaceae (*Foveoretitricolpites*), Combretaceae (*Pseudocolpopollis*), and Droseraceae (*Ornatetradites* and *Droseridites*) indicate a Miocene age for the Quilon beds. The spore and pollen assemblage recovered generally supports the Lower to Middle Miocene age assigned to Quilon beds, by the earlier workers on the basis of faunal evidence.

The spore and pollen assemblage along with the microthyriaceous fungal fruit bodies clearly points towards a tropical humid climate with plenty of rainfall during the Neogene period of Kerala.

The Neogene flora of the Quilon beds was essentially of the coastal type and indicates the presence of brackish water swamps and lagoons along the coast line.

## INTRODUCTION

**K**ING<sup>1</sup>, as early as 1882, classified the vast Tertiary sediments of the Kerala State in South India into the Lower Quilon beds consisting of fossiliferous limestones, carbonaceous clays, calcareous clays and sands, and the Upper Warkalli beds of variegated sands, white plastic clays, carbonaceous clays and associated seams of lignite. The entire Tertiary sequence of Kerala rests directly upon the Archaeans and itself is overlain by a variable thickness of recent to subrecent marine and estuarine sediments.

The Kerala Tertiaries extend all along the coast of that state almost continuously from Cape Comorin in the South to Manjeshwar, bordering the Mangalore District of Mysore in the North. They clearly reveal two major basins of deposition, viz., (1) between Trivandrum and Ponnani in the South including central Kerala, and (2) between Cannanore and Kasargod in North Kerala. The Quilon beds were originally believed to be of limited extent confined to the type locality at Padappakkara. Subsequently

Kumar and Pitchamuthu<sup>2</sup> traced the Quilon limestones towards Nedungulam, Paravur and Varkallai and Damodaran<sup>3</sup> located them at Edvai. Jacob and Sastry<sup>4</sup> identified this limestone in a bore hole from Chavara. Poulse and Narayanaswamy<sup>5</sup>, more recently, indicated that the marine calcareous beds spread over a considerable area from Varkallai in the South to Shertallai in the Northern part of Kerala under the cover of recent deposits.

The Quilon beds are best exposed in the southern basin at Padappakkara 11 Km north-east of Quilon and also at Nedungulam, Edvai, Paravur and in the drill holes north of Varkallai. The limestone is richly fossiliferous containing numerous, often beautifully preserved, specimens of foraminifera, corals, echinoids, molluscs, ostracods and crabs. In the northern basin, the Quilon beds are very insignificant and particularly located at the base of the sea cliff near Meenkunnu 6 Km north of Cannanore.

King<sup>1</sup>, who on lithological similarity considered the Warkalli beds overlying the Quilon's as equivalent to the Cuddalore sandstones of Tamil

Nadu, assigned a Middle Miocene age to the Quilon beds. Jacob and Sastry<sup>1</sup> on the basis of a study of foraminifera from a bore hole at Chavara assigned a Lower Miocene (Burdigalian) age to these beds. Subsequently Dey<sup>6</sup> from an exhaustive study of the molluscan fauna from Padappakkara considered the Quilon's to be of Middle Miocene (Vindobanian) age.

#### PALYNOLOGICAL RESULTS

Palynological investigations of the Kerala Tertiaries in general and the Quilon beds in particular have been very few and far between. The authors have undertaken this study with the express purpose of providing a comprehensive account of the palynological assemblages of the Quilon and Warkalli sediments so that they may be meaningfully utilized not only for stratigraphical purposes but also to unravel the floristic complexes and palaeoclimatical set up during the Neogene of Kerala and to assess and evaluate the nature of the depositional environment during this period. The following are the main results of the authors' study with regard to the Quilon sediments, the detailed account of which would be published elsewhere.

The material used in this study consists of a large number of samples of clay, often with many invertebrate remains in varying degrees of preservation, and darkish carbonaceous clays from the Quilon beds exposed at Padappakkara, Paravur and Edvai.

Palynologically the Quilon clays are found to be extremely rich. A number of hystrichosphaerids, acritarchs, and a galaxy of excellently preserved spores of pteridophytes and pollen grains of angiosperms and a fairly sizeable number of fungal fruit bodies and spores have been recovered from these clays by suitable maceration techniques. The angiosperm pollen grains constitute the predominant elements of the Quilon microflora. No gymnospermous pollen grains, either winged or unwinged, have been encountered in any of the large number of samples studied.

The pteridophytic spores are referable to Lycopodiaceae, Gleicheniaceae, Ophioglossaceae, Dicksoniaceae, Adiantaceae, Schizaeaceae, and Polypodiaceae. Of these, the spores of Polypodiaceae-Dicksoniaceae complex are profusely represented and next in the order of numerical importance are Lycopodiaceae and Schizaeaceae. The following are some of the important taxa recorded by the authors, viz., *Lygodiumsporites padappakkarensis* sp. nov., *Intrabaculisporis quilonense* sp. nov., *Gleichenidites cercinidites*, *Eximospora sparsus* sp. nov., *Verrucosisporites dakshinensis* sp. nov., *V. pulvinulatoideis*, *V. paravurensis* sp. nov., *Foveosporites raoi* sp. nov., *Foveotrilites bifurcatus*

sp. nov., *Lycopodiadites caperatus*, *Cibotidites kundavaensis*, *Cingulatisporites miocenicus* sp. nov., *Pteridacidites sahii* sp. nov., *Laevigatosporites ovatus*, *Polypodiisporites impariter*, *P. ornatus*, *P. perruatus*, *P. multiverrucatus*, and *Schizaeosporites multistriatus* sp. nov.

The angiosperms constituting the largest contingent of the Quilon microflora are represented by the pollen of both monocotyledons and dicotyledons. The monocotyledons are represented by the pollen of Potamogetonaceae (*Retipilonapites tertiarius* sp. nov., *R. arcotense*), Aroideae (*Spinainaperturites neogenicus* sp. nov., *Retialetes quilonensis* sp. nov.), Liliaceae (*Liliacidites padappakkarensis* sp. nov., *L. densireticulatus*), Palmae (*Palmaepollenites keralensis* sp. nov., *P. neyvelii*, *P. longisulcus* sp. nov., *Arecipites punctatus* sp. nov., *Verrumonocolpites indicus* sp. nov., *Couperipollis punctitectatus* sp. nov., *C. ellipticus* sp. nov., *Clavapalmaedites hammenii* gen. et sp. nov., *Echinosulcites ovatus* gen. et sp. nov., *Paravuripollis mulleri* gen. et sp. nov., *Edvapollis punctatus* gen. et sp. nov., *Longaperites hammenii* sp. nov., *Quilonipollenites sahii* gen. et sp. nov., *Dicolpopollis padappakkarensis* sp. nov., *D. minutus* sp. nov., *D. longicolpatus* sp. nov., and *Spinizonocolpites quilonensis* sp. nov.), Lemnaceae (*Spinamonoporites indicus* sp. nov.), and Gramineae (*Monoporopollenites gramineoides*). Of these the pollen of Palmae is very abundantly represented and shows significant resemblances with the pollen of the modern palms such as *Cocos*, *Hyphaene*, *Areca*, *Pinanga*, *Iriarte*, *Lepidocaryum*, *Nipa*, *Calamus* and *Metroxylon*.

Pollen grains referable to the following assemblage of the dicotyledonous families have been recognized in the Quilon beds, viz., Nymphaeaceae, Menispermaceae, Euphorbiaceae, Vitaceae, Combretaceae, Ebenaceae, Loranaceae, Dipterocarpaceae, Sapindaceae, Oleaceae, Lecythidaceae, Simarubaceae, Rubiaceae, Araliaceae, Symplocaceae, Anacardiaceae, Rhizophoraceae, Bombacaceae, Hippocrateaceae, Caesalpiniaceae, Aquifoliaceae, Nyssaceae, Caprifoliaceae, Compositae, Ctenolophonaceae, Labiateae, Meliaceae, Sapotaceae, Polygalaceae, Proteaceae, Haloragaceae, Sonneratiaceae, Olacaceae, Thymeliaceae, Moraceae and Droseraceae. The commonly represented taxa are, viz., *Crotonoidaepollenites euphorbioides* gen. et sp. nov., *Retitricolpites grandis* sp. nov., *R. americana*, *Retitrescolpites indicus* sp. nov., *Foveotricolpites piercei* sp. nov., *Crotonotricolpites densiclavatus* sp. nov., *Loranthipites elegans* gen. et sp. nov., *Margenipollis quilonensis* sp. nov., *M. kutchensis* comb. nov., *Ctenolophonidites costatus*, *Polycolpites granulatus*, *Pseudocolpopollis combreoides* gen. et sp. nov., *Cauveripollis superbus*, *Hippocrateaceadites quilonensis* sp. nov., *Zonocostites indicus* sp. nov., *Palaeocoprosmadites keralensis*



sp. nov., *Gothanipollis indicus* sp. nov., *Compositoipollenites argutus*, *Bombacacidites minutus* sp. nov., *Costatipollenites paucicornatus*, *Cupaniedites punctatus* sp. nov., *Margocolporites oligobrochatus*, *M. tsukadai*, *Sapotaceoidapollenites africanus*, *S. neyvelii*, *S. keralensis* sp. nov., *Meliapollis quilonensis* sp. nov., *Polygalacidites ovatus*, *Myricipites harrisii*, *Maculoporites quilonensis* sp. nov., *Verrutripurites perversicatus* sp. nov., *Tetrapollis quadrangularis* sp. nov., *T. ornatus* sp. nov., *Haloragacidites delicatus* sp. nov., *H. neyvelii*, *Parsonsidites conperi* sp. nov., *Anacolosidites luteoides*, *Clavaperiporites jacobii*, *Ornatetradites droseroides* gen. et sp. nov., and *Droseridites minor* sp. nov.

On the whole among the angiosperms the pollen of arborescent plants predominates over that of the herbaceous ones. The rarity of the pollen of Gramineae points towards the paucity of grass cover in the vegetation. The majority of the pollen grains exhibit various kinds of exine sculpturing, often of a very ornate type, which incidentally indicates that they were produced by entamophilous plants growing in and around the vicinity of the depositional basin and that there was not much of long distance transportation of the grains.

The hystrichosphaerids of the Quilon microflora consist of species of *Achmosphaera*, *Cleistosphaeridium*, *Hystrichosphaeridium*, *Spiniferites* etc., and the acritarchs include the species of *Baltisphaeridium*. The fungal fruit bodies are referable to microthyriaceous (ascomycetous) fungi.

The abundance of the spores of the Polypodiaceae-Dicksoniaceae complex, and the occurrence of *Pteridacidites*, *Intrabaculisporis*, *Cingulatisporites*, *Ciboridites* and the pollen grains of Compositae (*Compositoipollenites*), Sonneratiaceae (*Verrutripurites*), Dipterocarpaceae (*Foveoretitricolpites*), Combretaceae (*Pseudocolpopollis*), and Droseraceae (*Ornatetradites*, *Droseridites*) point towards the Miocene age for the Quilon beds. The Quilon microfloral assemblage is fairly comparable with the Miocene assemblages of the Eniwetok, Fiji, Bikini and Palau islands of the South Pacific area<sup>7</sup>, Neyveli lignite and Cauvery basin of Tamil Nadu<sup>8,9</sup>, Bengal basin<sup>10</sup>, and the Rusizi valley of Burundi<sup>11</sup>. While it is not possible at this stage to be specific as to the exact horizon of the Quilon beds within the Miocene age, the spore and pollen assemblage we have recovered generally supports the Lower to Middle Miocene age (Burdigalian to Vindobanian) assigned to these beds by the earlier workers on faunal evidence<sup>1-6,12</sup>.

#### CONSIDERATION OF PALAEOCLIMATE AND DEPOSITIONAL ENVIRONMENT

A overwhelming majority of the taxa with recognizable botanical affinities indicate the presence of either exclusively or chiefly tropical families in

the Quilon flora. Mention must be made in this connection of the occurrence of such families as Gleicheniaceae, Schizaceae, Polypodiaceae, Aroideae, Palmae, Lecythidaceae, Araliaceae, Symlocaceae, Rhizophoraceae, Sonneratiaceae, Bombacaceae, Hippocrateaceae, Caesalpiniaceae, Combretaceae, Sapotaceae, Dipterocarpaceae, Ctenolophonaceae, Meliaceae, Olacaceae, Anacardiaceae, and Droseraceae. The abundance of ferns and the presence of Dipterocarpaceae, Ctenolophonaceae, Olacaceae and *Duabanga* of Sonneratiaceae points towards heavy precipitation. It thus appears that the climate during the Neogene of Kerala was of the tropical humid type with plenty of rainfall. As the modern climate of Kerala is of the same kind, one is tempted to presume that perhaps there had not been much of a change in the climate of this region since the Neogene times.

A critical analysis of the diverse kinds of microfloral elements recorded clearly indicate that neritic, shallow marine to brackish water conditions prevailed during the deposition of the Quilon beds. The earlier faunal evidence also pointed to the same environment. There is no doubt that the Neogene Quilon flora was essentially of a coastal type. The record of pollen grains resembling such accredited mangrove taxa as *Barringtonia* of Lecythidaceae, *Rhizophora* of Rhizophoraceae, *Lumnitzera* of Combretaceae, *Nipa* and *Iriarteia* of Palmae clearly testifies to the presence of brackish water swamps and lagoons along the coast line. Present immediately interior to the mangrove belt of the vegetation there were probably a number of fresh water ponds dotting the landscape as evidenced by the occurrence of the pollen grains of Potamogetonaceae, Lemnaceae, Nymphaeaceae, and *Myriophyllum* of Haloragaceae.

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## LETTERS TO THE EDITOR

### EQUATORIAL SPREAD-F CONFIGURATIONS AND MAGNETIC ACTIVITY

THE morphological characteristics of equatorial spread-F have been extensively studied over the past two decades using published ionospheric data bulletins (Clemesha and Wright<sup>1</sup> and Skinner and Kelleher<sup>2</sup>, for earlier work). However, these investigations suffer from the drawback that they do not take into consideration, the type of spread-F, as this is not given in the published data. A better approach that is now being adopted<sup>3-4</sup> is the direct use of ionograms, usually quarter-hourly, for the study of equatorial spread-F.

Spread-F on equatorial ionograms usually manifests in two forms: range spread or equatorial type spread-F and frequency spread or temperate latitude-type spread-F. Range spread-F on ionograms is characterised by a general widening and diffusion of the F layer trace over the entire frequency range at which echoes are seen while in frequency spread-F the widening and diffusion of the trace is present at frequencies at and around the critical frequency ( $f_oF^2$ ). The mechanisms responsible for the origin of these two types of equatorial spread-F are considered to be different<sup>1</sup>. Recently King<sup>5</sup> claimed that spread-F, in general (including equatorial spread-F), is not due to partial reflection from irregularities but due to total reflection from rather sharp tilts in the isoionic contours and that frequency spread-F is the decay product of range spread-F. However, Skinner and Kelleher<sup>2</sup> argue that although oblique reflections from inclined isoionic contours could lead to a broadening of the F layer trace, it is not a major factor in producing equatorial spread-F. A very recent study made by us on equatorial spread-F configurations using quarter-hourly ionogram data of Kodaikanal for a six year period (1964-69) showed that both the range and the frequency spread-F configurations show a positive correlation with solar activity and the occurrence patterns of the same show a significant similarity<sup>6</sup>. In view of this observation, we further investigated another aspect of equatorial spread-F configurations, i.e., their occurrence in relation to magnetic activity, using quarter-hourly ionogram data of Kodaikanal (Geomag. Lat.  $0.6^\circ$  N; Dip  $3.5^\circ$ ), the results of which are present in this brief communication.

To investigate the dependence of the occurrence of the two types of spread-F configurations on magnetic

activity, days in each month for the entire period (January 1964-December 1969) are divided into those of quiet and disturbed depending on whether AP (magnetic character figure) is  $\leq 5$  or  $\geq 15$  respectively. This approach is followed in deviation to the alternative, often used, one of considering the "International Quiet" and 'disturbed' days in each month, as an 'International Quiet day' in some months may only be equal to a 'disturbed day' in other months. For each month, the quarter-hourly ionogram data of Kodaikanal for the two sets of days are examined for the presence of either of the two types of equatorial spread-F configurations. It is to be pointed out here that the rather unusual forms of spread-F that are known to exist on equatorial ionograms<sup>3-4</sup> are not taken into consideration in this study. From these data, the monthly percentage of each type of spread-F configuration is evaluated separately for both quiet and disturbed days. Median values of sunspot number corresponding to the quiet and disturbed days have also been obtained for each month. Running averages were then calculated to smooth out short term and seasonal variations. In Fig. 1 is shown the variation

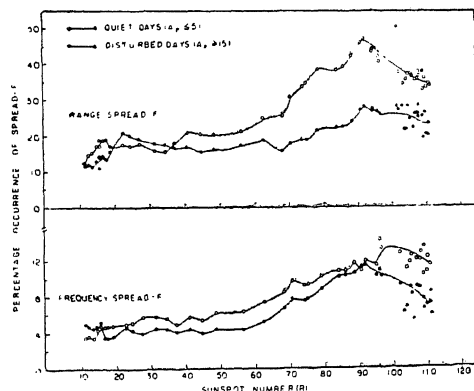


FIG. 1. Variation of percentage occurrence of range and frequency spread-F with sunspot number for quiet ( $A_p \leq 5$ ) and disturbed ( $A_p \geq 15$ ) conditions at Kodaikanal for the period 1964-69.

of the percentage occurrence of both the range and the frequency spread-F configurations with sunspot number, for quiet and disturbed days. It can be clearly seen from Fig. 1 that the effect of increased magnetic activity is to reduce the occurrence of

both range and frequency spread-F and this feature is more evident during moderate solar activity periods ( $R = 100$ ) than during low solar activity periods ( $R = 20$ ). This result differs from the work of Chandra and Rastogi<sup>7</sup> who reported that frequency spread-F does not exhibit significant reduction due to magnetic disturbances.

The above finding coupled with our earlier observations<sup>6</sup> on equatorial spread-F configurations suggest that the most common forms of equatorial spread-F may be due to a same causative mechanism, as the statistical behaviour (dependence on solar activity, magnetic activity and monthly occurrence pattern) of the two types are found to be very similar.

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# FRANK-CONDON FACTORS AND $r$ -CENTROIDS OF C-X SYSTEM OF MgII

BABCOCK<sup>1</sup> discovered the presence of MgII in the sunspot and solar disk spectra. Davis<sup>2</sup> in an exhaustive study of the spectrum of  $\beta$ -pegasi, a typical M type star giant, observed MgII to be an important constituent of the star. In view of the astrophysical importance of the spectrum of MgII, an attempt is made to compute Frank-Condon factors and  $r$ -centroids, for the  $C^2\pi-X^2\Sigma$  system (Khan<sup>3</sup>) using methods proposed by Fraser and Jarmain<sup>4</sup> and Nicholls and Jarmain<sup>5</sup> respectively. The overlap integrals for this system are evaluated by using Morse<sup>6</sup> potential function whose validity with respect to  $A^2\pi$  and  $X^2\Sigma$  states has already been established (Patel<sup>7</sup>).

The  $r$ -Centroids of the C-X system of MgII molecule are initially computed by graphical method.

The energy difference ( $U_{12}$ ) versus internuclear separation ( $r$ ) plot is observed to be a parabola, a rare case. The  $r$ -centroids are determined by using only an arc of the parabola as suggested by Nicholls<sup>1</sup>. It is interesting to note that the  $r$ -centroids for some of the bands (e.g., 2, 2 band) whose  $\sum_{v''} p_{v''}$  values lie outside the parabola could not be obtained. The  $r$ -centroids for the C-X system are also computed using quadratic method and the values are presented in Table I.

TABLE I  
Frank-Condon factors and  $r$ -centroids of C-X system of MgII molecule

| $v/v''$ |   | 0     | 1     | 2     |
|---------|---|-------|-------|-------|
| 0       | c | 0.940 | 0.058 | 0.002 |
|         | b | 1.737 | ...   | ...   |
|         | c | 1.737 | ...   | ...   |
| 1       | a | 0.058 | 0.813 | ...   |
|         | b | 2.158 | 1.800 | ...   |
|         | c | 2.159 | 1.800 | ...   |
| 2       | a | 0.001 | 0.124 | 0.672 |
|         | b | 2.744 | 2.203 | ...   |
|         | c | 2.743 | 2.204 | 1.861 |

(a) Frank-Condon factors.

(b)  $r$ -Centroids ( $\text{\AA}$ ) by graphical method.

(c)  $r$ -centroids ( $\text{\AA}$ ) by quadratic method.

Since  $aa/a$  is  $28.57\%$ , Frank-Condon factors are determined by the analytical method of Fraser and Jarmain with  $r$ -shift correction and are represented in Table I along with  $r$ -centroids. From the table, it can be observed that the  $v/v'' = 0$  sequence is the most intense one in accordance with the experimental observation.

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## N.Q.R. AND STRUCTURE OF CRYSTALLINE

 $\alpha$ ,  $\alpha'$ -DIBROMO-*m*-XYLENE

TABLE I

| Nucleus          | Locus | $\theta$ | $\phi$   | Bisector | $\theta$ | $\phi$   |
|------------------|-------|----------|----------|----------|----------|----------|
| $^{79}\text{Br}$ | 1     | 68° 0'   | 91° 40'  | Internal | 90° 20'  | 301° 10' |
|                  | 2     | 111° 30' | 150° 30' | External | 51° 0'   | 30° 50'  |

$\theta$  and  $\phi$  are the longitudinal and azimuthal angles referred to the growth axis of the crystal as the polar axis. The  $\phi = 0$  plane is chosen arbitrarily in the crystal.

angles to the growth axis, while the external bisector is inclined to it at an angle of 51°, it is more likely that the  $b$ -axis is parallel to the internal bisector in which case, the growth axis might be parallel to the  $a$  or  $c$  axes of the crystal.

The presence of only two loci and a single n.q.r. frequency for the compound gives rise to two possibilities, on the valid assumption that the Z-axis of the c.f.g. tensor lies along the C-Br bond direction. The structure of the molecule is  $\text{C}_6\text{H}_4(\text{CH}_2\text{Br})_2$ , and the two bromines replace one hydrogen each in the two  $\text{CH}_2$  groups attached to the two carbons in the 2 and 6 positions in a benzene ring. Since there is only a single n.q.r. frequency observed, both bromine nuclei must be present in chemically as well as crystallographically identical surroundings. Since there are only two loci and hence two orientations for the c.f.g. tensors (and hence for the C-Br bonds as well) either the two C-Br bonds in a molecule must be parallel to one another, there being two sets of molecules with their planes inclined to one another or the two C-Br bonds in a single molecule must be inclined to one another at an angle of 73°, with only a single set of molecules all parallel to one another. Mere experimental evidence is insufficient to logically eliminate either one of these possibilities. However, with all the molecules in the crystal parallel to one another, a cleavage plane parallel to the plane of the molecules is very likely to be present. The absence of any cleavage plane in the crystal therefore lends weight to the former alternative, namely, the presence of two sets of molecules with their planes inclined to one another, the two C-Br bonds in any single molecule being parallel to one another. A simple calculation based on a tetrahedral angle between the C-C and C-Br bonds indicates that in this case, the two C-Br bonds in a molecule lie in a plane perpendicular to the plane of the molecule or either side of it.

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ALTHOUGH there are two bromine atoms in the compound  $\alpha$ ,  $\alpha'$ -dibromo-*p*-xylene, there is only one n.q.r. line reported for this compound<sup>1</sup>. No data on n.q.r. frequencies were available in literature for the corresponding ortho and meta xylenes. According to the data given by Groth<sup>2</sup>, the *m* and *p*-xylenes crystals are monoclinic while the *o*-xylene is orthorhombic. An investigation in our laboratory with an externally quenched super-regenerative UHF pushpull oscillator described elsewhere<sup>3</sup> revealed only a single n.q.r. line at a frequency of 267.8 MHz at room temperature in the *m*-xylene while no signal could be obtained for the *o*-xylene. In order to ascertain more information about this interesting situation of the presence of only a single n.q.r. frequency, in spite of the existence of 2 bromines per molecule, an attempt is made to obtain the orientation of the different C-Br bonds in the crystal by studying the Zeeman effect of the n.q.r. lines in the compound  $\alpha$ ,  $\alpha'$ -dibromo-*m*-xylene. The compound is obtained from Messrs. Koch-Light Laboratories, Inc. Its melting point is 77° C. Single crystals of sufficiently large size could not be obtained from solution. However, cylindrical crystals of sufficient size were grown from melt using the Bridgmann technique. The oscillator is so mounted that the axis of the inductance coil is horizontal and lies at the centre of a pair of Helmholtz coils, which can be rotated about a vertical axis, supplying the d.c. magnetic field. The crystal, mounted in a crystal holder and placed within the inductance coil, can be rotated about the axis of the coil. By the combined rotation of the Helmholtz coils and the crystal, any orientation ( $\theta, \phi$ ) of the magnetic field relative to the crystal can be obtained.

The application of a weak magnetic field results in the splitting of the original n.q.r. line into two pairs of components. The inner pair merges for particular directions of the magnetic field which form a cone with its axis as the principal electric field gradient (c.f.g.) Z-axis. In this preliminary study, two loci are obtained. The directions of the principal c.f.g. Z-axes were calculated by the least squares method suggested by Bucchi and Cecchi<sup>4</sup>. The directions of the Z-axes of the c.f.g. tensor together with the internal and external bisectors are given in Table I. The angle between the two Z-axes is 73°.

A tabular form giving the number of equivalent directions and the relations among them for crystals of various symmetries has been published recently by J. L. Narayan *et al.*<sup>5</sup>. Comparison of the present observation with the table confirms that the crystal is monoclinic with one of the two bisectors parallel to the  $b$ -axis. Since the internal bisector is at right

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### GRAVIMETRIC DETERMINATION OF LANTHANUM WITH N-*p*-CHLOROPHENYL-*m*-NITROBENZOHYDROXAMIC ACID

A SUCCESSFUL quantitative gravimetric determination of lanthanum has been made in presence of several metal ions with N-*p*-chlorophenyl-*m*-nitrobenzohydroxamic acid (N-*p*-Ch-Ph-*m*-NBHA). The lanthanum forms canary yellow granular complex at pH 7.3 to 8.8 and the composition of the complex was found  $[C_{13}H_8O_4N_2Cl]_3 La$ .

The reagent was synthesized by the modified procedure of Tandon and Priyadarshini<sup>1</sup>.

A standard aqueous solution of lanthanum (III) as nitrate containing 2.78 to 13.90 mg and about 500 ml of double distilled water was taken in a litre beaker and heated to 60° C on a water bath; then 20 ml of reagent solution in ethanol were added dropwise with constant stirring. The pH of solution was raised gradually by adding dilute solution of ammonium hydroxide and the desired pH was adjusted by ammonium chloride solution. The canary yellow complex, so obtained, was digested for 3 hrs. over a water bath. The precipitate was filtered through a sintered glass crucible G 4 and washed thoroughly with hot water and finally with 20% aqueous ethanol. The complex was dried at 110° C and weighed directly as  $[C_{13}H_8O_4N_2Cl]_3 La$ .

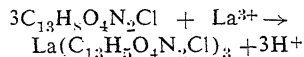
The results of a few estimations of various quantities of lanthanum indicated that 2.78 to 13.90 mg lanthanum could be determined with an accuracy of  $\pm 0.01$ .

Lanthanum could be separated from  $Ag^+$ ,  $Mn^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Ni^{2+}$ ,  $Cd^{2+}$ ,  $Hg^{2+}$  and  $Ga^{3+}$  by using 0.1% KCN solution as a masking agent and  $Pb^{2+}$ ,  $Pd^{2+}$ ,  $Sn^{2+}$ ,  $Sb^{3+}$ ,  $Bi^{3+}$ ,  $Zr^{4+}$  and  $Ti^{4+}$  by using 0.1% citrate and oxalate as masking agents.

Lanthanum could also be separated from  $Al^{3+}$ ,  $V^{5+}$  and  $Mo^{6+}$  by using Mg-EDTA as masking agent.

The I.R. spectra of the N-*p*-chlorophenyl-*m*-nitrobenzohydroxamic acid as mull showed the peaks at 3225, 1608 and 923  $cm^{-1}$  due to stretching vibrations of O—H, C=O and N—O respectively. After

the complex formation, spectrum does not show any peak due to O—H stretching vibration (3225  $cm^{-1}$ ). The absorption peak due to C=O stretching vibration is located at 1555  $cm^{-1}$ ; this lowering of carbonyl group frequency indicates the binding of carbonyl oxygen to the metal. The peak due to N—O stretching vibration is almost unaffected indicating that coordination is not through nitrogen. Based on the bidentate nature of the reagent, the complexation may be represented as



The reagent is superior to others in the case of preparation, solubility in ethanol and keeping qualities in the solid form. The composition of the complex is definite and thus it is directly weighable. Complex precipitation and easy filtration of the complex formed favour the use of this reagent as good gravimetric reagent for lanthanum. Low conversion factor (0.1419) is another advantage of this reagent. It may be advantageous therefore to replace the older reagents with this for the standardisation of mg amounts of lanthanum in solution. The method can successfully be applied for the gravimetric estimation of the lanthanides<sup>2</sup>.

The authors wish to express their sincere thanks to Shri K. G. Pilley, Principal, Government Polytechnic, Ujjain, Dr. M. M. Bokadia and Shri S. D. Choubey.

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### OCCURRENCE OF KAEMPFEROL AND ALOE-EMODIN IN THE LEAVES OF CASSIA ALATA LINN.

THE leaves of *C. alata* were shown to have laxative action<sup>1</sup>, antitumour activity<sup>2</sup> and insecticidal properties<sup>3</sup>. From the roots and seeds of this plant Tiwari *et al.*<sup>4,5</sup> reported the presence of  $\beta$ -sitosterol, chrysophanol, xanthones and two new anthraquinone pigments. The only report on the chemical constituents of the leaves of this plant was by Hauptmann *et al.*<sup>6</sup> who reported the isolation of rhein and a yellow dibasic acid.

In view of the reported medicinal properties a systematic chemical examination of the leaves of this plant has been taken up and is described hereunder.

The alcoholic extract of 2 kg of the leaves was concentrated and towards the end water was added. The aqueous suspension was then fractionated with petroleum ether, ether and ethyl-acetate to get the respective extracts.

The petroleum ether extract was saponified and chromatographed over alumina when only one crystalline component was obtained, shining plates from acetone, m.p. 139° and analysed for  $C_{29}H_{50}O$ ; acetate: m.p. 126°. These properties are in good agreement with those of  $\beta$ -sitosterol and its acetate. The identity was further confirmed by T.L.C. comparison with authentic  $\beta$ -sitosterol.

The ether extract gave strongly positive tests for flavonoids and anthraquinones. The residue was macerated with benzene when practically all anthraquinone positive substances were extracted. The remaining strongly flavonoid positive residue when crystallised twice from acetone gave fine yellow cluster needles, m.p. 272–75° and analysed for  $C_{15}H_{10}O_6$ ; m.p. 184–86°; methyl ether: 165–67°. The properties of the original substance and its derivatives agreed well with those of aempherol and its derivatives. The identity was established by m.m.p. and T.L.C. comparison with authentic kaempferol.

The benzene soluble fraction from the above showed three anthraquinone positive spots in T.L.C (chloroform-methanol 97:3 and ethyl acetate-methanol-water 100:16:14). This residue was separated into acid and neutral fractions.

The acid fraction on crystallisation from methanol yielded rhein, m.p. 315–17° (decomp.). Its identity was established by preparing the acetate, m.p. 242–44 (decomp.) and m.m.p. and T.L.C. comparison ethyl-acetate-methanol-water 100:16:14 with authentic rhein.

The neutral fraction on chromatography over silicagel yielded an amorphous anthraquinone positive substance which could not be crystallised and hence was not examined further. An orange-yellow crystalline compound, m.p. 223° was obtained from the benzene eluates. The compound gave a pink colour with 10% alcoholic alkali, a blood-red colour with conc. sulphuric acid and a deep orange colour with neutral ferric chloride solution. The compound analysed for  $C_{15}H_{10}O_5$ ; acetate: pale yellow needles from acetone, m.p. 172°; benzoate: pale yellow needles from acetone, m.p. 228°. The properties of the original compound and its derivatives agreed well with those of aloe-emodin and its derivatives. Its identity was further confirmed by m.m.p. and T.L.C. comparison (chloroform-methanol 97:3) with authentic aloe-emodin.

A small amount of brown powder has separated from the ethylacetate extract which showed the

presence of two components in T.L.C. The residue on oxidative hydrolysis (refluxed for half an hour with 1% aqueous methanolic HCl containing 1% ferric chloride at 98°) gave a positive colour reaction for anthraquinones. It is evident from this that the original substance is a mixture of reduced form of anthraquinones. Further work could not be done due to dearth of material.

Thus, from the leaves of the plant  $\beta$ -sitosterol, kaempferol, rhein and aloe-emodin were isolated, characterised and identified. Kaempferol and aloe-emodin are being reported for the first time from this plant.

Our thanks are due to Dr. H. Friedli, Sandoz Ltd., Switzerland, for gift of authentic samples of some anthraquinone derivatives. Our thanks are also due to Prof. L. R. Row, Head of the Department of Chemistry, Andhra University, for elemental analysis.

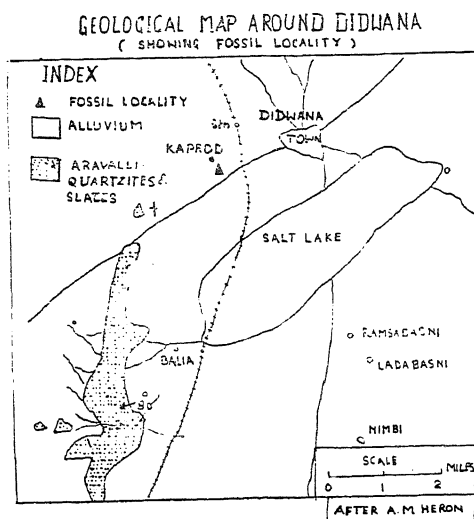
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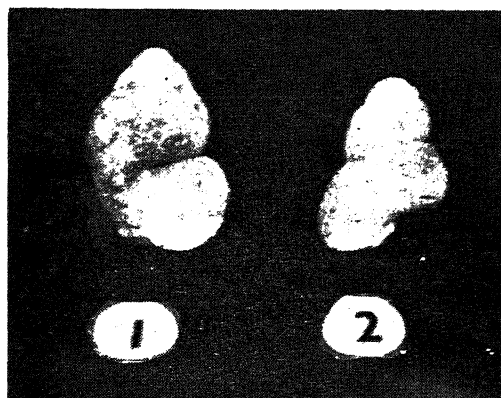
#### ON THE OCCURRENCE OF GASTROPOD FOSSILS IN THE KANKER DEPOSITS AROUND DIDWANA, RAJASTHAN

THE present note records for the first time, the occurrence of a few well preserved fresh water gastropod fossils of a species, the *Vivipara bengalensis* (Lamarck) collected from the grey coloured kanker deposits at a depth of 1.76 m from the surface in the village Kaprod, 3 Km west of Didwana 27° 24' : 74° 34' town, Rajasthan (Geographical Map around Didwana, showing fossil locality). The kanker deposits are bounded by an isolated spur, mapped as Aravallis by Heron<sup>1</sup>, have a wide horizontal distribution under a thin capping of sand, occurring also at a depth of 3.05 m from

the surface over the halite deposits in Didwana salt lake area discovered recently, by the author<sup>2</sup>, and has been considered to be more than 10,000 years old by Singh *et al.*<sup>3</sup>, based on carbon dating. Pascoe<sup>4</sup> also considers these kanker deposits belonging to the Older Alluvium of Holocene age.



The collected gastropod fossils of *V. bengalensis* (Lamarck); family Viviparidae, range in size from 14.2 mm to 22.3 mm in length and 11.4 mm to 15.5 mm in breadth; shells ovoidal, smooth, dextrally coiled with a thick periostracum; spire high consists of four rounded whorls; body whorl fairly large; aperture entire, ranging from 6.1 mm to 10 mm in height and 6.5 mm to 13.1 mm in breadth, (Figs. 1-2).



FIGS. 1-2. Fig. 1. Apertural view. Fig. 2. Side view,  $\times 1$ .

This fresh water gastropod fossil species, designated "*Phasepachydolichia*" by Annandale<sup>5</sup>, has been recorded by several workers<sup>5-7</sup>, from different parts

of the country. Based on his detailed work, Prashad<sup>8</sup> has pointed out, the importance of this species in tracing the evolutionary trend of the Group *V. bengalensis*. According to him "It appears certainly that the *V. bengalensis* Group was evolved from some species like *V. normalis* (Hislop) of the Vivipari Dissimiles" Group. Secondly the occurrence of this fresh water species in kanker deposits suggests that initially these sediments were deposited in fresh water conditions, thus strengthening the view held by Singh *et al.*, on the basis of palynological evidences, that the salt lake was a fresh water one, to begin with.

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#### RECORD OF NEW HOSTS OF *GANEQ TIGRINUM* (TREMATODA : LECITHODENDRIIDAE)

DURING a routine examination of reptiles and birds for helminths, two specimens of *Ganeq tigrinum* were obtained from the small intestine of a pied myna (*Sturnopastor contra*) at Gaya (Bihar). Several specimens of this trematode were also collected from the small intestine of a tortoise (*Kachuga dhorgoka*) from the same locality. The worms were well developed and contained numerous embryonated eggs. This trematode, first described by Mehra and Negi (1928)<sup>3</sup> and also reported from toad<sup>2</sup> and a reptile (*Chameleon zeylonicus*)<sup>4</sup> is a common intestinal parasite of frogs in northern India. With the exception of only one species, *Ganeq gobindia*<sup>1</sup>, the other eleven species of *Ganeq* are all intestinal parasites of anuran amphibia. The occurrence of the trematode genus *Ganeq* in an avian and a chelonian host is recorded here for the first time.

It is now clear that *Ganeo* has a wider host range than what was believed earlier. The occurrence of this genus in a bird is interesting from the point of view of host specificity, because it is strange how a natural parasite of poikilothermous hosts could grow into a well developed, egg-producing adult in a bird. Parasitization of a definitive host involves successful entry of the infective stage. It is believed that the infective stage of *Ganeo* enters the definitive host through some intermediate agent, probably an insect, which is ingested by it. In the present case, the entry of the infective stage of the trematode into the pied myna through insects feeding on mollusc tissue, appears quite likely, as the bird is one of open cultivation and is chiefly insectivorous, feeding on caterpillars and all types of insects from amongst roots of grass. Furthermore, it appears as if the biochemical and biophysical demands of *Ganeo* are not very strict and are fulfilled in the intestine of any vertebrate. Its occurrence in fish, amphibian, reptilian as well as bird intestines suggests that its enzyme system works well over a wide range of temperature and its body proteins are less antagonistic, evoking almost no host response.

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### GLUTAMATE DEHYDROGENASE ACTIVITY IN THE NORMAL AND DENERVATED GASTRO- CNEMIUS MUSCLES OF FROG *RANA HEXADACTYLA*

PROGRESSIVE decrease of oxidative and glycolytic activity<sup>1-4</sup> and an increased proteolytic activity<sup>5-6</sup> are the prominent features of myopathies such as nutritional and hereditary muscular dystrophies and the atrophy resulting from disuse, denervation and tenotomy. Under these conditions if the carbohydrate and protein metabolisms are altered, the glutamate dehydrogenase, which catalyzes a key reaction in these metabolic pathways, may be affected. Hence, an attempt is made in the present investigation to study the activity pattern of this enzyme in the denervated muscle,

*Rana hexadactyla* of medium size were denervated by sciatic nerve section under aseptic conditions. The frogs were fed *ad lib* with cockroaches. After 1, 2, 3 and 4 weeks post-operatively, the animals were sacrificed, both the denervated and the contra-lateral control gastrocnemius muscles were excised quickly. 10% (W/V) homogenates of the tissues were prepared in 0.25 M sucrose and centrifuged at 2,500 rpm for 15 min., 0.4 ml of each supernatant (containing 40 mg tissue) was assayed for the glutamate dehydrogenase (GDH E.C. 1.4.1.3) activity by the method of Lee and Lardy (1965)<sup>7</sup>. Protein levels were determined by the method of Lowry *et al.* (1951)<sup>8</sup>.

Progressive decrease of GDH activity was found in the muscle after denervation, the decrease being 37% after four weeks (Table I). Similar decrease in GDH activity has been reported earlier in the rat hemidiaphragm<sup>9</sup> and in the pedipalpal muscle of scorpion<sup>10</sup>. The decrease in the activity of GDH may be due to the disintegration of mitochondria, since fragmentation of fiber mitochondria has been reported in the atrophied muscle<sup>11</sup>. Further, GDH has been shown to be sensitive to the levels of NADH *in vitro*<sup>12</sup>. A high concentration of NADH leads to a decrease of GDH activity<sup>13</sup>. So it can be presumed that a prominent role might be played by NADH in the inactivation of GDH; for it is known that the ratio of NADH/NAD increases in the skeletal muscles of genetically dystrophic and vitamin E deficient animals<sup>1, 12</sup>. Present results suggest that a similar phenomenon might happen in denervated muscle also.

The substrate concentration velocity relationships of the enzyme showed a decreased maximal velocity ( $V_{max}$ ) and increased Michaelis-Menten constant ( $K_m$ ) (Table I). The NAD dependent activity of the enzyme also revealed that on denervation, the  $V_{max}$  value decreased and  $K_m$  increased showing a general deterioration in the affinity of the denervated muscle enzyme with both the substrate and coenzyme. However, the enzyme of both normal and denervated muscles had a lower  $K_m$  value for NAD than for glutamate, indicating preferential binding of coenzyme with the enzyme.

The detoxification of ammonia is mainly achieved either by the formation of glutamine from glutamate (catalyzed by glutamine synthetase activity) or by the formation of glutamate from the keto acids catalyzed by GDH. Glutamine synthetase activity was not detected in the amphibian gastrocnemius muscle (Unpublished observation of Prameelamma) and the GDH activity was also low in the denervated muscle indicating less mobilization of ammonia. Hence it can be assumed that the glutamate deaminating activity by GDH in the denervated muscle is decreased



TABLE I

GDH activity, expressed in  $\mu$  moles of formazan/mg. protein/hr.

|                   |    | 1st week             | 2nd week             | 3rd week             | 4th week             | Kinetic parameters of GDH activity |               |                              |               |
|-------------------|----|----------------------|----------------------|----------------------|----------------------|------------------------------------|---------------|------------------------------|---------------|
|                   |    |                      |                      |                      |                      | GLUTAMATE                          |               | NAD                          |               |
|                   |    |                      |                      |                      |                      | $V_{\max}$<br>( $\mu$ Moles)       | $K_m$<br>(mM) | $V_{\max}$<br>( $\mu$ Moles) | $K_m$<br>(mM) |
| Normal Muscle     | .. | 0.112<br>$\pm 0.010$ | 0.097<br>$\pm 0.012$ | 0.099<br>$\pm 0.015$ | 0.102<br>$\pm 0.020$ | 0.092                              | 0.464         | 0.08                         | 0.005         |
| Denervated Muscle | .. | 0.103<br>$\pm 0.011$ | 0.077<br>$\pm 0.012$ | 0.073<br>$\pm 0.011$ | 0.064<br>$\pm 0.014$ | 0.061                              | 0.740         | 0.053                        | 0.015         |
| % Decrease        | .. | 8%                   | 20%                  | 23%                  | 37%                  |                                    |               |                              |               |
|                   |    | $p > 0.05$           | $p > 0.05$           | $p > 0.02$           | $p > 0.01$           |                                    |               |                              |               |

so that this enzyme system may not add further ammonia toxicity to the already existing ammonia.

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#### CUTICULAR ADAPTATION IN A COPEPOD PARASITE *PENNELLA ELEGANS*

PREVIOUS work on Pennellids show that the cephalothorax is modified into a narrow cylindrical and often coiled structure, known as 'neck', without a carapace, unlike in closely related free living types like caligids, which possess a broad, flat cephalothorax covered with a carapace<sup>1-3</sup>. Such a modification has been suggested by Wilson<sup>2</sup> as an adaptation to enable the parasite to penetrate into the host's flesh. It is not known what structural and chemical peculiarities of the cuticle of this neck region allow for the flexibility of the parasite and the present investigation is an attempt in this direction.

The thin outer membrane corresponding to the epicuticle in *Pinnella elegans* is homogeneous and stains deep blue with Mallory's triple stain<sup>4</sup>. This condition is in contrast to the reports of the earlier workers on the decapod crustaceans in which the epicuticle stained red with Mallory<sup>5-6</sup> due to the presence of fuchsinophilic protein considered by Dennell and Malek<sup>7</sup>, and Krishnan<sup>8</sup> as the precursor for tanning.

The epicuticular protein of the cuticle of decapod crustaceans has been reported to be positive to xanthoproteic and Millon's tests<sup>9</sup>. In *Pennella elegans*, the cuticle of the neck is negative to these tests. Ferric chloride and argentaffin tests, indicative of phenols, are also negative<sup>10</sup>. In decapods, the epicuticle and the outer regions of the procuticle which would undergo tanning show the presence of fuchsinophil protein together with diphenols and an oxidase involved in the oxidation of diphenols to

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quinones. The negative results obtained with catechol test in the cuticle of neck may indicate the absence of phenol oxidase<sup>11</sup>. The epicuticle reacts only to the Biuret test indicating the presence of the simple protein<sup>12</sup> as in the inner endocuticle of decapod crustaceans. It may therefore be inferred that the chemical constitution of the cuticle of the neck region is such that it cannot be tanned.

The procuticle, in addition to the possession of horizontal lamellations reported in previous work, shows transverse patches of denser material alternating with the regions which are light staining in *Pennella elegans* (Fig. 1). The denser regions stain deep blue and the lighter region light blue in Mallory. This pattern of the procuticle is unlike

fixed by the horns in the tissues of the host, the necessary flexibility needed for the animal seems to be provided by the cuticle of the neck region.

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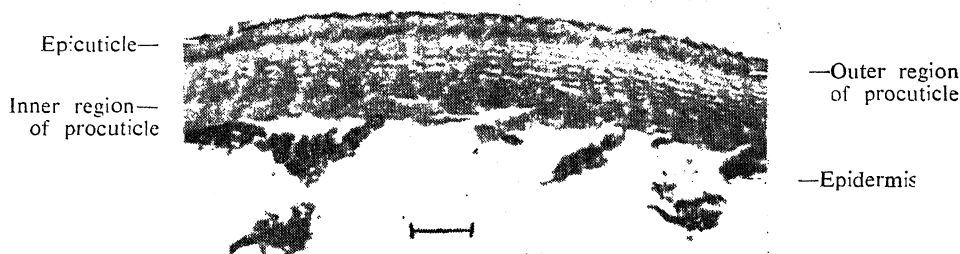


FIG. 1. Transverse section through the cuticle of the neck of *Pennella elegans*, stained in Mallory's triple stain.

any reported in other arthropods so far studied. Another feature which may be significant is that the entire procuticle also reacts only to Biuret test as the epicuticle<sup>12</sup>.

Chromatographic analysis for amino acids of the cuticle of the neck following the method of Giri and Rao<sup>13</sup> showed 13 amino acids, namely, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, proline, serine and threonine and absence of aromatic amino acids and sulphur containing amino acids which are involved in the hardening of the cuticle.

The absence of any trace of hardening, the presence of a simple protein forming the basal matrix of the procuticle and the structural peculiarities noted in the procuticle may suggest that all these contribute to flexibility of the cuticle. This is significant in the light of the observations reported by Quidor<sup>14</sup> and Wilson<sup>2</sup> that it is the neck region that functions in a Corkscrew like fashion enabling the parasite to penetrate deeper into the tissues of the host. With the head region

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## A VIRULENT BIOTYPE OF RACE 77 OF LEAF RUST OF WHEAT AND ITS SOURCES OF RESISTANCE

A CONVENTIONAL approach in the identification of rust races lies in the use of standard differential which, however, are not ideal for the detection of biotypes within races. Stakman<sup>3</sup> emphasised the need of the inclusion of additional varieties in the standard differentials for this purpose. Accordingly, 10 additional varieties (UP 215, UP 319, WG 357, NI 5439, HD 4502, HS 1138-6-4, Kalyansona, Moti, Sharbati sonora and Sonalika) were used in the differential set for the identification of the races and the biotypes of leaf rust of wheat.

During the crop year 1973-74, a collection of leaf rust of wheat on variety CC 62 from Dharwar (Karnataka) on analysis yielded race 77 which produced infection type "4" on wheat variety, NI 5439 being resistant to type race 77, reported from India<sup>4</sup> in 1955. Later the test isolate was met with in several samples of important wheat varieties.

Single spores were picked up from susceptible type of pustule developed on NI 5439 and inoculated on Agra local wheat (one spore per leaf). Single pustules as developed were increased separately. Each single spore culture of the test isolate was analysed on the differential set<sup>1</sup> along with the additional varieties mentioned above. It was observed that each single spore culture produced identical infection on each variety of the set and the disease reactions were in conformity with those produced by the test isolate.

One of the single spore culture of the test isolate was further compared with type race 77 for infection types produced on the differential set. The two differed in their pathogenicity. Var. NI 5439 was susceptible to the test isolate and resistant to the type race 77; because of the difference in their pathogenicity the test isolate has been designated as 77-A and deposited in the Type Culture Collection at this laboratory.

In all, 11 samples yielded the test virulence. Nine out of 11 samples were from Tamil Nadu and one each from Karnataka and Madhya Pradesh. In the very first year of its appearance its frequency was 1.7% as against 15.0% frequency of type race 77. The fact that the test isolate was met with in several samples from Tamil Nadu, indicates that, probably, its original home is somewhere in the Nilgiris. The original home of race 104 of leaf rust has been reported to be in Nepal hills<sup>2</sup>. It is apparent, therefore, that the foci of infection of leaf rust of wheat exist both in Nepal hills in the north and Nilgiri hills in the south.

The appearance of virulent biotype 77-A made it obligatory to have information on the resistant donors which can be exploited in the breeding programme. Since race 77 is quite virulent and is quite widespread it is, therefore, desirable to have information on the resistant donors against both the virulences, i.e., race 77 and biotype 77-A. The wheat varieties (181) were tested in the seedling stage and the information on resistant donors is as follows.

### *Resistant Donors Against Race 77*

NI 5439, HD 2012, HD 2099, HP 1102, L 104, L 117, L 118, L 122, L 127, L 128, L 144, Tanori 71, Yogin 53, and Nadodores; all these cultivars were susceptible to biotype 77-A.

### *Resistant Donors Against Race 77 and Biotype 77-A*

HD numbers 1739, 1928, 1999, 4502, 4530, H, 7484, HI 7628, Raj 911, WL 1002, HS 38, VL 4171, NS 879/4, N 5749, IWP 500, IWP 503, HW 153, MP 112, MPO 193, Anzas, Burgas-2, Gaza and Yuma.

There are a number of varieties which are resistant to type race 77 but these are susceptible to biotype 77-A. A number of cultivars resistant to both the virulences were also identified. These cultivars would be useful in the hybridization programme because these can confer resistance against both virulences. Cultivars, resistant to all the known virulences of leaf rust in the country, were also identified. These are IWP 500, IWP 503, HW 153, NS 879/4 and Burgas-2.

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|                              |                 |
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**ELECTROPHORETIC VARIATION IN  
HAEMOLYMPH PROTEINS OF THE TOBACCO  
CATERPILLAR, *SPODOPTERA LITURA* (F.)  
INFECTED WITH A NUCLEOPOLYHEDROSIS  
VIRUS**

NUCLEOPOLYHEDROSES cause considerable derangement in the physiology of infected insects. Changes in the protein and the amino acid metabolism have been reported in several cases<sup>1</sup>. In the present paper, changes occurring in the electrophoretic pattern of haemolymph proteins in the case of nucleopolyhedrosis virus infected larvae of *Spodoptera litura* are presented.

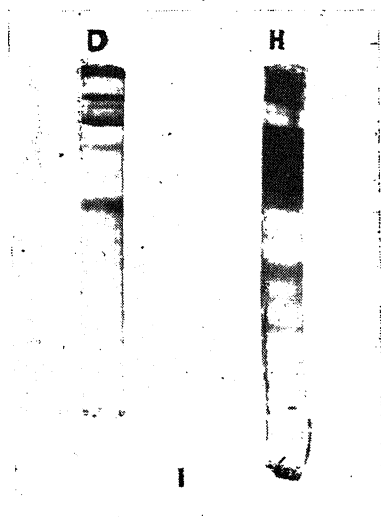


FIG. 1. Disc electrophoretic pattern of haemolymph of healthy (H) and diseased (D) larvae of *Spodoptera litura* (F.).

Freshly moulted fourth instar larvae of *S. litura* were infected by feeding them on castor leaf disc spotted with 10  $\mu$ l of virus suspension containing  $4 \times 10^6$  inclusion bodies. The larvae were reared individually and inactivated in chips of ice, and 10  $\mu$ l of the blood was removed by clipping off, the first pair of prolegs. Whole blood from each insect was diluted with 100  $\mu$ l of 40% sucrose. Ten  $\mu$ l of diluted blood was used for disc electrophoretic separation using acrylamide gel according to the method of B. J. Davis<sup>2</sup>. The current supplied was 3 mA per tube at 100 V for 60 minutes. Amido-Schwartz 1% in 7% acetic acid was used to stain the gels and 7% acetic acid v/v was used for destaining and preservation. Electrophoretic pattern of haemolymph protein of diseased larvae during advanced stage, i.e., two days prior to death was compared with that of the healthy individuals.

The haemolymph of healthy *S. litura* larvae showed thirteen protein bands with poorly stained diffused bands at the lower end of the column and well-stained concentrated bands at the top (Fig. 1). The latter two extensively stained and broad bands at the top appear to be a combination of two or more protein fractions. In the case of larvae infected with a nucleopolyhedrosis virus, a decrease in the concentration of the two main slow moving protein fractions and almost total depletion of the other protein bands has been observed (Fig. 1). This result is in close conformity with the earlier reports<sup>3, 4</sup>. The staining intensity of the various bands by Amido-Schwartz is indicative of the relative protein concentration. From the pattern developed, it is apparent that there are a few complex bands with similar mobilities and character. This should be subjected to further separation.

Since fat body, (the primary tissue infected by the nucleopolyhedrosis virus of all lepidopterus insects) is the site of synthesis of haemolymph proteins<sup>5, 6</sup>, the decrease in the total protein may be due to suppression of host protein synthesis and/or its degradation because of severe functional lesions of the fat body.

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**A NOTE ON NEMATODE FUNGAL COMPLEX IN  
CROSSANDRA (*CROSSANDRA UNDULAEFOLIA*  
SALISB.) IN COIMBATORE**

IN recent years a marked decline in the cultivation of Crossandra in and around Coimbatore, Tamil Nadu, has been noticed. The seedlings do not establish well on transplanting. The grown-up plants appear chlorotic and exhibit wilt symptoms in many areas. Survey of Crossandra fields was therefore carried out

to ascertain any possible nematode-fungal complex on this crop. Infested soil and root samples were collected from different localities. Centrifugal flotation technique<sup>2</sup> was adopted for the nematode recovery.

The examination of soil and root samples have shown the presence of two nematode species *Pratylenchus delattrei* and *Helicotylenchus dihystra* consistently in association with crossandra. The population of *Pratylenchus* ranged from 95 to 286 per gm of root and 83 to 524 per 250 cc of soil respectively. Similarly, the population of the spiral nematode, *Helicotylenchus*, ranged from 5 to 269 per gm of roots and 22 to 238 per 250 cc of soil. Species of other genera, *Rotylenchulus*, *Hoplolaimus*, *Tylenchorhynchus* and *Xiphinema* were noticed in negligible numbers in one or two localities only. The roots with distinct lesions, when plated on agar medium, yielded *Fusarium solani*.

Reports of association of *Pratylenchus* spp. with the *Fusarium* spp. causing wilt diseases in lucerne<sup>1</sup>, pea<sup>3</sup>, etc., and *Helicotylenchus multicinctus* and *Rhizoctonia* complex on banana<sup>4</sup>, and *H. dihystra* and *Phytophthora cinnamomi* on pine<sup>4</sup> are well known. A similar association may be present in the decline of Crossandra around Coimbatore.

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## MATERNAL INFLUENCE ON BACTERIAL LEAF BLIGHT REACTION IN RICE

THE influence of maternal parent on the expression of bacterial leaf blight disease, incited by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, in rice has not been studied in detail so far. Ratho *et al.* (1975) from their diallel analysis studies, reported the presence of maternal effect and other reciprocal differences for both tillering stage as well as boot leaf stage reactions of this disease<sup>2</sup>. The present paper reports the reciprocal differences in respect of 21 crosses and their reciprocals tested at tillering and boot leaf stages of plant growth.

The parents used in the present study were, Taina-3 mut. 587-4, (T. 3 mut) Lacrosse × Zenith-Nira (LZ-Nira), Wase aikoku-3, the resistant donors, Tkm-6 the moderately resistant and Ambemohor, Ratna and Padma the susceptible parents. Crosses were made in a complete diallelic pattern. The 42 F<sub>1</sub>'s along with the parents were grown in two sets, each in a complete randomised block design with two replications under high nitrogen fertilization (100 kg N/ha). The plants were clip inoculated (one set at tillering stage and the other at boot leaf stage) with the bacterial cell suspension (ca. 10<sup>8</sup> cells/ml) prepared from a 48 hr old culture of a virulent isolate (Isolate-I) of *X. oryzae* grown on potato sucrose agar medium. The length of lesion developed below the point of inoculation was measured on the 15th day after inoculation. Significant reciprocal difference in disease reaction was estimated for each cross by F test where

$$F = \frac{\text{Larger variance}}{\text{Smaller variance}} \text{ of the cross and its reciprocal involved.}$$

The data on the presence of significant reciprocal difference in different crosses are presented in Table I. Among the 21 cross combinations, significant reciprocal differences could be detected only in six crosses at tillering stage and eleven at boot leaf stage, out of which four were common for both the stages. The cross combinations LZ-Nira × Wase aikoku-3, LZ-Nira × T. 3 mut., Wase aikoku-3 × T. 3 mut. and Tkm-6 × Padma exhibited significant reciprocal differences at both the stages. The first three crosses involved resistant × resistant parents while the last one was between moderately resistant and susceptible parents. The crosses involving Ratna as the susceptible parent in Ratna × T. 3 mut. and Ratna × LZ-Nira had significant differences only at tillering stage. Hence, it is seen that, of all the crosses involving resistant parents, the one between moderately resistant × susceptible parents and the other two between the resistant × susceptible parents showed significant differences only at tillering stage. Highly signi-

TABLE I  
Reciprocal differences for bacterial leaf blight reaction in different crosses

| Cross                      | Tillering stage   |                        |        | Boot leaf stage   |                        |         |
|----------------------------|-------------------|------------------------|--------|-------------------|------------------------|---------|
|                            | Variance of cross | Variance of reciprocal | F      | Variance of cross | Variance of reciprocal | F       |
| Ambemohor × LZ-Nira        | 4.75              | 2.87                   | 1.66   | 17.71             | 1.31                   | 13.52+  |
| Ambemohor × Wase aikoku-3  | 3.65              | 3.73                   | 1.02   | 33.20             | 35.00                  | 1.05    |
| Ambemohor × Tkm-6          | 5.29              | 3.05                   | 1.73   | 5.81              | 25.49                  | 4.39*   |
| Ambemohor × Ratna ..       | 7.87              | 2.35                   | 3.35   | 1.76              | 8.15                   | 4.63    |
| Ambemohor × Padma          | 0.59              | 0.44                   | 1.34   | 0.23              | 0.18                   | 1.28    |
| Ambemohor × T. 3 mut.      | 4.75              | 2.11                   | 2.25   | 19.41             | 20.74                  | 1.07    |
| LZ-Nira × Wase .. aikoku-3 | 0.64              | 13.06                  | 20.41+ | 0.70              | 14.97                  | 21.39+  |
| LZ-Nira × Tkm-6 ..         | 11.68             | 3.29                   | 3.55   | 39.02             | 0.88                   | 44.34+  |
| LZ-Nira × Ratna ..         | 6.37              | 19.87                  | 3.12*  | 16.70             | 9.47                   | 1.76    |
| LZ-Nira × Padma ..         | 20.64             | 20.90                  | 1.01   | 142.72            | 0.60                   | 237.87+ |
| LZ-Nira × T. 3 mut. ..     | 0.54              | 6.94                   | 12.85* | 26.81             | 5.51                   | 4.87+   |
| Wase aikoku-3 × Tkm-6      | 1.85              | 1.65                   | 1.12   | 23.98             | 22.87                  | 1.05    |
| Wase aikoku-3 × Ratna      | 2.76              | 0.65                   | 4.25   | 5.82              | 2.02                   | 2.88    |
| Wase aikoku-3 × Padma      | 9.10              | 8.89                   | 1.02   | 24.14             | 24.32                  | 1.01    |
| Wase aikoku-3 × T. 3 mut.  | 1.92              | 0.09                   | 21.33+ | 1.44              | 6.21                   | 4.31*   |
| Tkm-6 × Ratna ..           | 1.32              | 1.33                   | 1.01   | 128.64            | 32.97                  | 3.90*   |
| Tkm-6 × Padma ..           | 3.87              | 0.05                   | 77.40+ | 83.57             | 1.81                   | 46.17+  |
| Tkm-6 × T. 3 mut. ..       | 1.87              | 3.50                   | 1.87   | 1.84              | 12.01                  | 6.53*   |
| Ratna × Padma ..           | 3.69              | 6.95                   | 1.88   | 1.23              | 10.77                  | 8.75+   |
| Ratna × T. 3 mut. ..       | 5.54              | 0.81                   | 6.84+  | 17.21             | 7.58                   | 2.27    |
| Padma × T. 3 mut. ..       | 8.66              | 7.34                   | 1.18   | 45.36             | 47.98                  | 1.06    |

\* and + Significant at 5% and 1% levels respectively.

ificant reciprocal differences were observed for the cross Tkm-6 × Padma followed by Wase aikoku-3 × T. 3 mut. and LZ-Nira × Wase aikoku-3.

Crosses involving resistant × moderately resistant parents such as Tkm-6 × T. 3 mut. and LZ-Nira × Tkm-6 exhibited significant difference at boot leaf stage only. Similarly among the resistance × susceptible combinations such reciprocal differences were seen only in two cross combinations (Ambemohor × LZ-Nira and Padma × LZ-Nira). Among the moderately resistant × susceptible combinations, besides the cross Tkm-6 × Padma being common to both the stages, Ambemohor × Tkm-6 and Tkm-6 × Ratna exhibited significant differences in reciprocal

crosses. One cross combination involving susceptible × susceptible parents (Ratna × Padma) showed the presence of reciprocal difference at boot leaf stage only whereas there was none at tillering stage. Highly significant reciprocal difference was observed in the cross combination LZ-Nira × Padma at boot leaf stage which did not reveal any difference at tillering stage.

Rath and Padmanabhan (1972) observed significant reciprocal differences for the type of lesion, number of lesion and reaction to blast disease of rice in certain cross combinations<sup>1</sup>. Data obtained from the present study provide adequate information suggesting that reciprocal differences do exist at least in

certain cross combinations and maternal parents influence the expression of disease reaction to a detectable degree.

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#### POWDERY MILDEW RESISTANCE IN PEA (*PISUM SATIVUM* L.)

POWDERY mildew is a very serious disease of pea caused by *Erysiphe polygoni* D.C. It has become a serious threat to pea cultivation in India. Vasudeva<sup>4</sup> estimated the losses caused by this disease to the tune of 23% but during years of epidemic, these losses increase enormously. Although effective chemical control measures are known, yet evolution of some suitable resistant variety is essential. Different pea workers Harland<sup>1</sup>, Heringa *et al.*<sup>2</sup> and Pierce<sup>3</sup> have tried to develop varieties with adequate resistance to powdery mildew. Present investigation was taken up to study the mode of inheritance of resistance to this disease and also to evolve resistant and high yielding varieties of pea.

Six hundred and fifty-five strains of pea were screened in the year 1970-71 and six strains were isolated, which possessed 95% resistance, viz., T 10, P 185, P 388, 6583, 6587 and 6588 which showed similar resistance in subsequent years as well, but being table pea with wrinkled seed, they were found inferior in yield as compared with the field variety T 163, which was found to be highly susceptible. In order to transfer resistance to the field pea, three crosses, viz., (T 10 × T 163), (P 185 × T 163) and (6583 × T 163) were made during 1972-73.

The F<sub>1</sub>'s were raised during 1973-74 both under controlled and uncontrolled conditions. For the control of the disease, four sprayings of sulfur (Sulfex) at 2.5 Kg per hectare were given at an interval of seven days, soon after flowering stage. Under uncontrolled conditions, the epidemic of the disease was created at adult stage by shaking

spore-bearing parts of pea plants over the population to be tested, followed by application of a fine spray of water in the evening. All the F<sub>1</sub>'s were found to be seriously affected. In F<sub>2</sub> grown during 1974-75, the number of susceptible and resistant plants was recorded in each of the three crosses and their ratios were tested by Chi-square test.

The F<sub>1</sub> progeny of T 10 × T 163 during 1973-74 was found completely susceptible while data given in Table I showed that the F<sub>2</sub> population segregated in ratio of 3 susceptible and 1 resistant. This clearly indicated that the susceptibility is controlled by a single dominant gene.

TABLE I

Segregation in F<sub>2</sub> generation of crosses  
susceptible × resistant

| Crosses          | Number of plants in F <sub>2</sub> |           | Chi-square<br>(3 : 1) | P         |
|------------------|------------------------------------|-----------|-----------------------|-----------|
|                  | Susceptible                        | Resistant |                       |           |
| 1. T 10 × T 163  | 336                                | 133       | 2.826                 | 0.10-0.05 |
| 2. P 185 × T 163 | 571                                | 165       | 2.616                 | 0.20-0.10 |
| 3. 6583 × T 163  | 500                                | 167       | 0.001                 | 0.98-0.95 |
| Pooled           | 1407                               | 465       | 0.025                 | 0.90-0.80 |

The two other crossed populations behaved similarly. F<sub>1</sub>s in both the populations, viz., (i) P 185 × T 163 and (ii) 6583 × T 163 were found to be completely susceptible while F<sub>2</sub> gave a good fit to a 3 : 1 ratio where resistance was found to be due to monogenic recessive homozygous condition. These findings are in conformity with those of earlier workers<sup>1-3</sup>. Thus present findings reveal that powdery mildew resistance in pea is a monogenic recessive trait.

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**EUPHORBIA GENICULATA LINN.  
A NEW HOST RECORD FOR  
SCLEROTIUM ROLFSSII (SACC.) GUR.**

DURING July, 1975, a serious stem and root rot disease of groundnut caused by *Sclerotium rolfsii* was observed at Gorakhpur specially in the fields with high moisture due to rains. Apart from groundnut, plants of *Euphorbia geniculata* growing in the field as weed, were found to be severely infected with the same fungus showing almost similar symptoms, i.e., stem with necrotic zones bearing white superficial mycelium and sclerotia generally aggregated along the affected stem portion of the plants.

Repeated isolations made on potato dextrose agar yielded *Sclerotium rolfsii*. Pathogenicity was established by inoculating many stems and branches of the plants with mycelial bits on one week old culture and by incubating them in a humid chamber at room temperature  $26 \pm 3^\circ \text{C}$ . Initiation of the symptoms were observed after 30 hours of incubation. Severe rotting of the affected plant parts with profuse mycelial growth and abundant sclerotial initials was encountered after 50 hours.

*Sclerotium rolfsii* causes diseases of many economically important plants. *Euphorbia geniculata* can be thought to act as an alternate host and dormant sclerotia perennating on the infected plant debris may provide inocula for the recurrence of the disease. Although, *Sclerotium rolfsii* has been reported on several plants from India,<sup>1-4</sup> it is a new host record for the fungus from this country. The transfer of the culture is being deposited in C.M.I., Kew, England.

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**IN VITRO PRODUCTION OF INDOLE ACETIC ACID BY CERCOSPORA CITRULLINA CKE.**

MANY plant pathogens have been known to synthesise Indole acetic acid (IAA) from tryptophan. IAA synthesis by leaf spot pathogens like *Helminthosporium oryzae* and *Pyricularia oryzae* and its participation in the disease development has been suggested by several workers<sup>4,6</sup>. Leaf spots caused by species of *Cercospora* and their ability to synthesise IAA has not been worked out in detail. The present study deals with the ability of *Cercospora citrullina* Cke., the leaf spot pathogen of muskmelon, to synthesise IAA from tryptophan.

Fifty ml of Czapek's medium were mixed with 0.1% of tryptophan in 250 ml Erlenmeyer flasks, autoclaved and inoculated with 8 mm discs of actively growing fungus obtained from a 7-day old culture on Czapek's agar. The flasks were incubated in the dark for 10, 20, 30 and 40 days at  $28 \pm 2^\circ \text{C}$ . At the end of the incubation periods, the mycelium was filtered through a Whatman No. 44 filter-paper, previously dried at  $100^\circ \text{C}$  to a constant weight. The weight of the mycelium was determined after drying at  $100^\circ \text{C}$  for 25 hrs. The culture filtrate was centrifuged at 2,000 g for 20 min to remove the spores. The pH of the culture filtrate was maintained at 3 by the addition of  $\text{NHCl}$ . IAA was extracted from culture filtrate with equal volumes of the peroxide free ether at  $4^\circ \text{C}$  with three solvent changes at 8 hr intervals. The ether fraction were evaporated at  $40^\circ \text{C}$  and the residue was dissolved in 2 ml of distilled methanol. IAA was detected chromatographically. An aliquot of 100  $\mu\text{l}$  of the methanol residue and the authentic sample of IAA were spotted separately on Whatman No. 1 filter paper and developed ascendingly in iso-propanol : ammonia : water :: 10 : 1 : 1 (v/v) in the dark<sup>1</sup>. The strips were air dried and sprayed with Salkowski reagent<sup>2</sup> to locate the auxin. Quantitative estimation of IAA was done employing the method of Gordon and Paley<sup>3</sup> using Salper's reagent. The mycelial growth and the quantity of IAA synthesised are presented in Table I.

TABLE I

| Days of incubation | Mycelial dry weight (mg/100 ml) | IAA synthesised (mg/L) |
|--------------------|---------------------------------|------------------------|
| 10                 | 140                             | 24.5                   |
| 20                 | 582                             | 27.5                   |
| 30                 | 455                             | 27.1                   |
| 40                 | 423                             | 24.1                   |

*Cercospora citrullina* Cke. synthesised large quantities of IAA only in the tryptophan added



medium. IAA production and mycelial growth were maximum on the 20th day. Later the growth and IAA synthesis decreased with the age of the culture. There appears to be a good correlation between mycelial growth and IAA synthesis. Since the fungus synthesised large quantities of IAA *in vitro*, similar synthesis is possible in infected leaves of muskmelon which may ultimately have an impact on the physiological processes like pectin and phenol metabolism as well as respiration.

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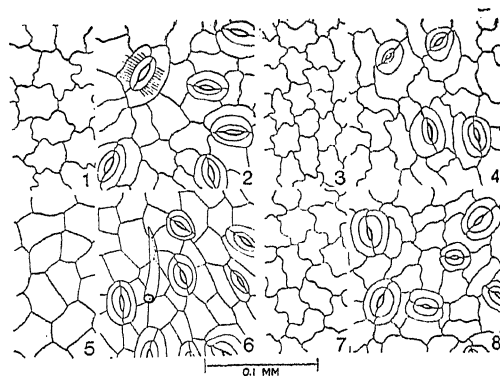
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### EPIDERMAL STUDIES IN *CINCHONA* (RUBIACEAE)

THE epidermal characters of four species of *Cinchona*, viz., *C. hybrida* Hort. ex Sasaki, *C. ledgeriana* Moens. ex Trimen, *C. officinalis* Linn., *C. succirubra* Pav. ex Klotzsch and one hybrid (*C. ledgeriana* × *succirubra*) have been studied particularly with a view to checking up the relationship of these characters in parents and their hybrid.

The epidermal cells are invariably irregular with usually sinuous (Figs. 1-4, 7, 8) or occasionally straight or arched anticlinal walls (Figs. 5, 6; *C. succirubra*). The leaves are hypostomatic. The stomata are evenly scattered on the intercoastal area and are irregularly oriented. They are invariably of rubiaceous or paracytic type having two subsidiary cells and one or more encircling cells lying parallel to the guard-cells (Figs. 2, 4, 6, 8). The latter are typically kidney-shaped with differential cell-walls. The size of the guard cells varies not only in different species but also in the same leaf of a species. The trichomes have been observed in all the species. They are confined to the lower surface with the exception of *C. succirubra* where they are present both on the upper and lower surfaces. They are invariably

non-glandular, short, unicellular, thin-walled (Fig. 6) and are scattered on the coastal as well as the intercoastal areas. Cuticular striations are present on the surface of epidermal cells in *C. hybrida* and *C. officinalis*. The striae flow out in two lateral groups from outer walls of the guard cells of some stomata, and run for a short distance on the surface of epidermal cells (Fig. 2). However, in *C. hybrida*, the striae also flow out all around from the base of the trichome.



FIGS. 1-8. Epidermal structures in *Cinchona* species. Figs. 1-2. Upper and lower epidermis respectively of *C. officinalis*. Figs. 3-4. Upper and lower epidermis respectively of *C. ledgeriana*. Figs. 5-6. Upper and lower epidermis respectively of *C. succirubra*. Figs. 7-8. Upper and lower epidermis respectively of the hybrid (*C. ledgeriana* × *succirubra*).

Epidermal cells per square mm, frequency of stomata, average size of stomata and stomatal index for species are given in Table I.

TABLE I

| Sl. No. | Name of species                             | No. of epidermal cells per sq mm |               | Frequency of stomata per sq mm | Average size of stomata in $\mu$ (L & B) | Stomatal index |
|---------|---|----------------------------------|---------------|--------------------------------|--|----------------|
|         |   | Upper surface                    | Lower surface |                                |  |                |
| 1.      | <i>C. hybrida</i>                           | 1024                             | 1344          | 380                            | 11.2<br>× 7.0                            | 22.04          |
| 2.      | <i>C. ledgeriana</i>                        | 1600                             | 880           | 288                            | 11.4<br>× 6.5                            | 24.65          |
| 3.      | <i>C. officinalis</i>                       | 1180                             | 1360          | 320                            | 14.0<br>× 7.0                            | 19.04          |
| 4.      | <i>C. succirubra</i>                        | 1192                             | 1200          | 496                            | 11.9<br>× 7.0                            | 29.24          |
| 5.      | <i>C. ledgeriana</i><br>× <i>succirubra</i> | 1448                             | 976           | 292                            | 11.7<br>× 7.7                            | 23.02          |

All values represent average of ten readings.

It is interesting to note that the hybrid (*C. ledgeriana* × *succirubra*) show certain epidermal

characters such as the number of epidermal cells per square mm, the shape of the epidermal cells, number of stomata per square mm intermediate to *C. ledgeriana* and *C. succirubra*. While the epidermal cells have nearly sinuous anticlinal wall in *C. ledgeriana* (Figs. 3, 4) and straight or arched walls in *C. succirubra* (Figs. 5, 6), they are slightly sinuous in the hybrid of these two species (Figs. 7, 8).

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# EFFECT OF HEAT TREATMENT AND GA FEEDING ON SEEDLING GROWTH OF MUNG

SUGE<sup>1</sup> AND TAKAHASHI *et al.*<sup>2</sup> reported beneficial effect of temperature and gibberellic acid on mesocotyle elongation of paddy seedlings and plants. On the contrary, high temperature imposed dormancy on some seeds<sup>3-4</sup>. In view of such conflicting reports, it was considered of interest to study the effect of high temperature on germination of mung. This should be interesting in view of the fact that germination of mung seeds is favoured, when atmospheric temperature is high (Summer) and delayed during cold season.

Uniform, air dried seeds of *Phaseolus aureus*, Rcxh. Cv. Pusa Baishakhi were placed in an oven at 40°, 50°, 60°, 70°, 80° and 90° C for one hour. The control and the heat treated seeds—10 each—were germinated in 5 ml distilled water (DW) and 250 ppm GA<sub>3</sub> solution in Petridishes on Whatman Filter-Paper No. 1. Germination was carried out in darkness and at room temperature (30 ± 2° C). At 96 hours of germination, lengths of root, hypocotyle and epicotyle were measured. In all, 5 germination sets were run and the results were consistent.

Data on root length are given in Table I. Root length of seedling in DW was enhanced by heat treatment, the maximum enhancement was caused at 80° C, while at 70° C it was slightly enhanced. Above 80° C, the root growth was considerably diminished. GA<sub>3</sub>, however, retarded the root length.

Hypocotyle elongation was slightly affected by the treatment in seedlings in DW. GA feeding enhanced hypocotyle elongation. Temperature above 80° C retarded hypocotyle elongation.

Epicotyle elongation was not much affected by heat treatment below 80° C in DW, and above this, the elongation was reduced. GA considerably enhanced epicotyle length.

It is thus seen that response to heat treatment by root, hypocotyle and epicotyle was variable. In a number of paddy varieties, Inouye and Ito<sup>5</sup> similarly

TABLE I

| Germination              | Feed-<br>ing<br>media | Control<br>(un-<br>heated) | 40° C         | 50° C         | 60° C         | 70° C         | 80° C         | 90° C        |
|--------------------------|-----------------------|----------------------------|---------------|---------------|---------------|---------------|---------------|--------------|
| Root length cm (a)       | D.W.                  | 10.0<br>± 0.8              | 11.4<br>± 0.5 | 11.5<br>± 0.1 | 11.5<br>± 0.3 | 10.3<br>± 0.3 | 13.1<br>± 0.5 | 3.5<br>± 0.6 |
|                          | G.A.                  | 8.2<br>± 0.6               | 7.3<br>± 0.4  | 8.0<br>± 0.4  | 8.2<br>± 0.4  | 8.1<br>± 0.7  | 6.2<br>± 0.7  | 4.5<br>± 0.6 |
| Hypocotyle length cm (b) | D.W.                  | 12.3<br>± 0.3              | 12.4<br>± 0.4 | 11.4<br>± 0.4 | 12.4<br>± 0.4 | 12.0<br>± 0.8 | 10.0<br>± 1.0 | 7.3<br>± 0.9 |
|                          | G.A.                  | 13.1<br>± 0.7              | 12.8<br>± 0.5 | 14.3<br>± 0.7 | 14.1<br>± 0.7 | 13.1<br>± 0.7 | 10.9<br>± 0.6 | 5.0<br>± 0.1 |
| Epicotyle length cms (c) | D.W.                  | 5.9<br>± 0.4               | 6.3<br>± 0.5  | 4.8<br>± 0.6  | 5.8<br>± 0.5  | 6.0<br>± 0.6  | 4.3<br>± 0.7  | 1.7<br>± 0.9 |
|                          | G.A.                  | 8.7<br>± 0.5               | 9.3<br>± 0.5  | 7.8<br>± 0.5  | 7.6<br>± 0.5  | 7.0<br>± 0.4  | 5.4<br>± 0.5  | 2.1<br>± 0.5 |

reported variable responses to heat pretreatment by root, mesocotyle and coleoptile. GA caused retardation of root length, while epicotyle length was promoted by GA. Data also showed that mung seeds can tolerate temperature upto 70° C as cotton seeds<sup>6</sup>, but above 70° C seedlings showed retarded growth. As Suge<sup>1</sup> remarked, mechanism of heat pretreatment is not known. But both heat treatment and GA may increase sensitivity to ethylene, CO<sub>2</sub> or may increase production of these or any other endogenous chemicals<sup>1</sup>, which cause enhanced length; alternatively, it may cause increased cell multiplication which may increase the length<sup>7</sup>.

Retardation or inhibition observed at 90° C may be due to denaturation of proteins and enzyme proteins; and if these are denatured, GA which is enzyme; mobilizing hormone also becomes ineffective.

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#### LITTLE KNOWN FEATURES IN THE FOLIAR EPIDERMOLGY OF SOME EUPHORBACEAE

SUBSEQUENT to Metcalfe and Chalk's<sup>1</sup> classic summary of the general anatomical features, and apart from the foliar anatomical study of 150 species of the single genus *Euphorbia*,<sup>2,3</sup> little has been added to our knowledge about the foliar epidermology of the Euphorbiaceae. The present paper brings into light some hitherto little known features in this regard. The occurrence of cuticular striations, cytoplasmic connections between the guard cells of the adjacent stomata on the one hand, guard cell and the epidermal cell on the other foliar sclereids, tannin-filled cells and mucilaginous epidermal cells are some of the

obscurely known features recorded here in several members of the family.

Santos<sup>4</sup> appears to be the first to report the occurrence of cuticular striations in *Excoecaria agallocha* Linn., followed by a reference to the same by Paliwal and Kakker<sup>2</sup> in the species of *Euphorbia*. In the present study, cuticular striations occur conspicuously in 7 species belonging to 6 genera and 3 tribes of the family. Nair and Maitreyi<sup>5</sup> described the leaves of *Sebastiania chamaelea* (L.) Muell. Arg. as hypostomatic and failed to record any striations. Contrary to this report, our study revealed that the leaves of *S. Chamaelea*, materials of which collected from different population samples of Visakhapatnam District, are not only amphistomatic but characterized by conspicuous presence of cuticular striations on both the adaxial and abaxial surfaces (Fig. B). The striations are seen better in a focus different from that of the epidermal cells. In the costal cells, they are prominent and run parallel to the longitudinal axis (Fig. D), whereas in the intercostal cells, the striae usually radiate from trichome bases (Fig. G), from the outer faces of the guard cells (Figs. C, G), rarely from the polar ends; encircling the guard cells (Fig. A) and pervade in all directions, along different angles and diverse perspectives, being continuous (Figs. C and D) or discontinuous and diffuse (Fig. F), or parallel (Figs. A, D, G) or reticulate or corrugated (Fig. B). The architecture of striations varies from species to species studied (Figs. A-C, G).

Ahmad<sup>6</sup> emphasized the systematic importance of the striations in the species of *Cestrum* and Krishnamurthy and Sundaram<sup>7</sup> described them in their studies on the pharmacognosy of Asclepiadaceae. Recently Srivastava<sup>8</sup> concluded, that the nature and distribution of striations vary from species to species in *Jasminum*. The diversity in the profiles of the striations, based upon the present study, is summarized in Table I.

An interesting feature, recorded for the first time in Euphorbiaceae, is the existence of cytoplasmic connections between the guard cells of the juxtaposed stomata and the epidermal cells, an aspect that finds no mention in all the earlier comprehensive anatomical treatises on the family. We observed cytoplasmic connections, which simulate miniature conjugation tubes, between the guard cells of side by side stomata (Fig. 1), and between guard cell and the adjacent epidermal cell (Fig. J), as explained in Table II.

Such cytoplasmic connections have earlier been baserved in a few taxa of angiosperm families other than Euphorbiaceae, such as Araliaceae,<sup>9</sup> in a few members of Amaryllidaceae<sup>10</sup> and in species of *Capsicum*<sup>11</sup> and *Asparagus*<sup>12</sup>.

TABLE I

| Sl. No. | Name of species                                       | Tribe          | Profiles of cuticular striations in the intercostal area |                             |
|---------|---|----------------|--|-----------------------------|
|         |   |                | Abaxial  | Adaxial                     |
| 1.      | <i>Ricinus communis</i> Linn.                         | .. Acalypheae  | P  | P                           |
| 2.      | <i>Tragia involucrata</i> Linn.                       | .. do.         | P  | P, D                        |
| 3.      | <i>T. involucrata</i> L. var. <i>cannabin</i> a Linn. | .. do.         | P<br>less prominent                                      | P, D<br>less prominent      |
| 4.      | <i>Trewia nudiflora</i> Linn.                         | .. do.         | P  | P, D<br>less prominent      |
| 5.      | <i>Jatropha panduraefolia</i> Andr.                   | .. Jatrophaeae | P  | D, P<br>slightly corrugated |
| 6.      | <i>Excoecaria agallocha</i> Linn.                     | .. Hippomaneae | P  | Not prominent               |
| 7.      | <i>Sebastiania chamaelea</i> (L.) Muell.-Arg.         | .. do.         | C, R   | C<br>less reticulate        |

D, Diffuse and discontinuous; C, Corrugated; P, Parallel; R, Reticulate.

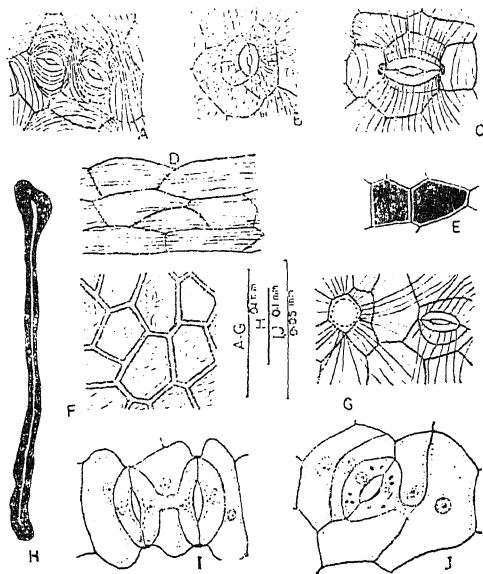
TABLE II

| Sl. No. | Name of species                       | Tribes      | Cytoplasmic connections  |                                       |
|---------|---------------------------------------|-------------|--------------------------|---------------------------------------|
|         |                                       |             | Between adjacent stomata | Between guard cell and epidermal cell |
| 1.      | <i>Croton bonplandianum</i> Baill ..  | Crotoneae   | +                        | -                                     |
| 2.      | <i>Codiaeum variegatum</i> Bl. ..     | Cluytieae   | +                        | -                                     |
| 3.      | <i>Euphorbia geniculata</i> Orteg. .. | Euphorbieae | +                        | -                                     |
| 4.      | <i>E. milli</i> Boiss ..              | do.         | -                        | +                                     |

+ Present;

- Absent

Foliar sclereids were met with in *Tragia involucrata* (Fig. H), which conform to some patterns illustrated by Foster<sup>13</sup> in several species of the genus *Mouriria* of Melastomataceae. Further, Ananda Rao and Bhupal<sup>14</sup> emphasized the significance of the foliar sclereids as tools of systematic value within a genus or in solving problems of disputed synonymy. Earlier,



FIGS. A-J. Figs. A, D, F. *Jatropha pandurifolia*. Fig. A. Abaxial surface with striations encircling the guard cells. Fig. D. Costal cells with parallel striations. Fig. F. Adaxial surface with diffuse and discontinuous, slightly corrugated striations. Fig. B. *Sebastiania chamaelea*. Abaxial surface with corrugated and reticulate striations. Fig. C. *Excoecaria agallocha*. Abaxial side with striae radiating from the outer faces of guard cells. Fig. E. *Phyllanthus rotundifolius*, tannin-filled cells. Figs. G, H. *Tragia involucrata*. Fig. G. Adaxial view with trichome and stoma as loci for radiating striations. Fig. H. A macrosclereid. Fig. I. *Croton bonplandianum*, Cytoplasmic connection between adjacent stomata. Fig. J. *Euphorbia milli*, Cytoplasmic connection between guard cell of the stoma and the adjacent epidermal cell.

in Euphorbiaceae, Webster<sup>15</sup>, in the New World species of *Phyllanthus*, described foliar sclereids of

'terminal origin' and Kakkar and Paliwal<sup>3</sup> reported 'racheoid idoblasts' in the species of *Euphorbia*.

We further observed tannin-filled cells in the epidermis of *Phyllanthus rotundifolius* Klein. (Fig. E), *Cleistanthus collinus* Benth., mucilaginous cells in *Acalypha ciliata* Forsk. and *A. indica* Linn. prismatic or rhombic crystals in the veins of *Chorisandra pinnata* Wt., *Emblica officinalis* Gaertn. and *Cleistanthus collinus* and druses in *Phyllanthus debilis* Herb. Ham. and *Jatropha gossypifolia* Linn.

The distinct distribution of these features in different species can provide data of supplementary systematic value to a limited extent.

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## REVIEWS AND NOTICES OF BOOKS

**Particles, Sources and Fields (Vol. 2).** By J. Schwinger. (Addison Wesley Publishing Company, Reading, Massachusetts), 1973. Pp. 459. Price \$ 18.50.

This is the second volume in the series of three by Prof. J. Schwinger on Particles, sources and fields. The present volume is devoted entirely to Electrodynamics.

Quantum electrodynamics underwent enormous developments in the last 30 years and the main architects have been Prof. Tomonaga of Japan and Professors Schwinger and Feynman of the U.S.A. While the techniques of Professors Tomonaga and Schwinger were similar, Feynman developed the diagrammatic representation of the perturbation terms and owing to its visual appeal, it has been extremely fashionable among theoretical physicists. In fact Feynman's techniques have been found useful even in non-relativistic manybody theory which is being widely used in solid state theory.

In the last 10 years, Prof. Schwinger has developed his theory of particles, sources and fields. His aim is to emphasize the unity of high energy particle physics, electrodynamics, gravitational theory and many-particle phenomena. According to him, his approach is intermediate between the operator field theory and S-matrix theory. The ideas depend heavily on quantum mechanics and relativity. While it is formulated in space-time, with corresponding use of momentum description (which is inevitable), his approach does not take for granted that infinite momentum space is involved right from the start. As he remarks, it is an extrapolation upward in momentum and downward in space and time keeping in contact always with experiment to test his theory. His source theory is associated with particle creation and annihilation and is connected with the instrument in question involved in the measurements. The source of a particular particle has thus embraced all the dynamical properties and mechanisms for creating the particle. While in the first volume he has developed the conceptual foundations and the mathematical apparatus of his new theory of particles, sources and fields, the succeeding 2 volumes deal entirely with the applications to electrodynamics, such as charged particle propagation, photon propagation, scattering of light by light and the relativistic theory of spin 0 and spin  $\frac{1}{2}$  particles etc. All these are very advanced topics and it will be useful to persons wanting to make use of these techniques for their research purposes. At present there are very few

theoreticians who are making use of the techniques developed by Prof. Schwinger. However, researchers who master this technique may find it extremely powerful in solving practical problems of physical phenomena.

The book under review contains very detailed calculations and is mathematically rich. In fact I am impressed by the calculations given in the book right down to arithmetical numbers. A few equations are reproduced below :

$$(a) \frac{1}{12} + \frac{3}{24} + \frac{11}{24} \quad (4-16-53)$$

$$(b) \frac{\pi^2}{4} \left( \frac{9}{40} + \frac{1}{9} \right) + \frac{1}{4} \left( \frac{3}{5} + \frac{7}{48} \right) = 1.016, \quad (5-4-207)$$

There is no doubt that this series of books will enrich any library of a scientific institute. Furthermore, particle physicists will slowly turn to these techniques and I recommend this book to all advanced research students and workers in this field. However, before reading Volume II, they are advised to master Volume I. K. P. SINHA.

**Creation and Detection of the Excited State (Vol. 3).** Edited by William R. Ware (Marcel Dekker, Inc., 270, Madison Avenue, New York, N.Y. 10016), 1975. Pp. viii + 193. Price \$ 23.50.

The series "Creation and Detection of the Excited State" is intended, according to the editor Dr. Ware, as a source of information on experimental techniques applicable to the study of all aspects of the formation and behaviour of excited molecules. Any book or series with such a goal should be welcome irrespective of the field, in view of the fast changing pace of experimental research. This series is particularly welcome, since a knowledge of the excited state characteristics of atoms and molecules is an important prerequisite for making opto-electronic devices such as lasers.

There are four chapters in the book and the first chapter is entitled "Experimental Methods in Phosphorescence-Microwave Double Resonance (PMDR)". In essence it is concerned with the methodology of detecting optically the magnetic resonances in various kinds of atoms and molecules. The utility of PMDR techniques in understanding the characteristic properties of triplet state in Phosphorescent aromatic as well as other kinds of molecules is discussed in detail in this chapter.

The second chapter is on the Detection of Transient Free Radicals by Electron Spin Resonance

**Spectroscopy.** The creation by flash photolysis of free radicals and the study by optical absorption spectroscopy of their properties have become very important endeavours in the fields of photochemical and photophysical reactions. Recently Electron Spin Resonance (ESR) techniques are being employed for the characterisation of flash photolysis products and the author of this chapter has discussed lucidly the methodology behind the application of ESR techniques in the detection of transient free radicals. The author has brought out clearly the fact that ESR spectroscopy has a definite place in the study of flash photolysis products.

The third and fourth chapters are concerned with two very important laser sources. The third chapter is devoted to the discussion of picosecond optical pulse generation and their applications. Amongst the various kinds of laser sources now available, the picosecond laser is the most sophisticated optical source with a wide ranging applications. The author has rightly pointed out that picosecond laser is a tempermental source. Despite the many advances made during the last few years by many workers in this field, the picosecond pulse generation remains more an art even to-day. The author has discussed the picosecond laser pulse generation and their application at a very practical level in this chapter.

Dye laser is a tunable source of coherent optical radiation and hence it is bound to attract the attention of research workers interested in a tunable source. There exist now-a-days modelocked dye lasers which can put out optical pulses of picosecond duration. The combined qualities of short pulse duration and tunability makes a dye laser a very versatile source. The authors of the fourth chapter on dye lasers have discussed clearly and concisely the basic physics and the performance characteristics of various types of dye lasers. Also, they have discussed the results from several experimental investigations in areas such as spectroscopy where the dye lasers have been used.

In his preface the editor mentioned: "The chapters aim at providing some one new to a particular area with sufficient information to permit establishing a particular technique in his own laboratory, given hardware and the normal skills of an experimental list." In my opinion the authors of the various chapters of this book have co-operated admirably with the editor in realising his above mentioned aim.

The print of the book is very pleasing and if the price also is equally pleasing I am sure the book will attract many customers.

S. V. PAPPU.

**Fifty Years of Research—Cotton Technological Research Laboratory, Bombay.** Edited by V. Sundaram. (Cotton Tech. Res. Lab., Anderwala Road, Matunga Road, Bombay 400 019), 1924–1974. Pp. xiv + 282. Price not given.

During the last 50 years cotton development in India has made significant strides both in the quality and variety of cottons as well as yield per acre. This has been of great benefit not only to the agriculturist but to the textile industry which has been consuming cotton. It is an example of how when knowledge of science is systematically and consistently applied, great progress can be made in commercial production. In this process, the Cotton Technological Laboratory has been of great value to the agricultural scientist in evaluating the experimental cottons, in ensuring purity of commercial varieties and detecting any deteriorations that might take place over a period. The Golden Jubilee of such a laboratory is therefore of considerable importance both for the scientific and for the commercial world.

To commemorate the occasion of its 50th anniversary or Golden Jubilee (1924–1974), the Cotton Technological Research Laboratory has published a new 282 page book, summarising in a systematic manner the work done by the Institute to date in 17 Chapters.

The topics dealt in various chapters concerns improvement in cotton quality and production, effect of agricultural practices, pre-cleaning and ginning, fibre properties, spinning, yarn characteristics, relation between fibre properties and spinning performance, fibre structure, moisture relations, effect of storage, neppiness of Indian cottons, chemistry of cotton and cellulose, cellulose degradation, cotton seed analysis, etc. There has been some overlapping information in some chapters. This is inevitable, as many of the investigations dealt with under different chapters are inter-related.

A review of the book shows that the laboratory has made significant progress both in the quantity and quality of work done since its inception. Facilities of the laboratory have been kept upto date by the addition of modern scientific equipments developed in India and abroad. Research facilities include X-ray unit, Electron Microscope and infra-red spectrometer in addition to several less sophisticated equipments.

It is really interesting to wade through the volume which contains summaries of more than 600 research publications, covering almost every aspect of cotton research. The volume will be quite interesting and useful to students, and research workers on cotton all over the country.

K. S.

# Current Science

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# BETA SPECTRUM OF $^{175}\text{Yb}$

P. MALLIKHARJUNA RAO, B. MALLIKARJUNA RAO, C. NARASIMHA RAO, RAJAN MATHEWS  
AND K. VENKATA REDDY

Laboratories for Nuclear Research, Andhra University, Visakhapatnam 530003

## ABSTRACT

The study of  $^{175}\text{Yb}$  is interesting from the point of view of Nilsson model, as this nucleus lies in the deformed region  $150 \leq A \leq 190$ . The shape data on the  $7/2^- \rightarrow 9/2^+$  beta transition of  $^{175}\text{Yb}$  is scanty and any attempt to measure the same by the usual singles method results in large uncertainties because of the presence of the conversion electron lines in the same energy region besides the weak intensity ( $\approx 3\%$ ) of the transition. The present work aims at a reasonably good estimate of the shape of the beta group in  $^{175}\text{Yb}$  employing a coincidence beta ray spectrometer which eliminates the interferences.

## 1. INTRODUCTION

THE decay scheme of  $^{175}\text{Yb}$  has been studied by many investigators<sup>1-5</sup>. The inner beta with an end-point energy of 353 keV, which is of present interest, is a non-unique first-forbidden transition ( $7/2^- \rightarrow 9/2^+$ ) with a high log ft value (7.48). Cork *et al.*<sup>1</sup> measured the beta spectrum of  $^{175}\text{Yb}$  with a double focussing magnetic spectrometer and reported that after subtraction of the high energy component ( $W_0 = 466$  keV) there was rather a large scatter in the points of the residual Kurie plot. A least-square fit to these points, in regions where no interference from internal conversion lines was expected, gave a beta component with  $W_0 = 374 \pm 30$  keV whose intensity was about 253 times that of the high energy component. Mize *et al.*<sup>2</sup> conducted beta-gamma coincidence experiments in which scintillation spectrometers were employed, (a bare Pilot plastic scintillator- $^{11}\text{B}$  as  $\beta$ -detector and NaI (TI) as  $\gamma$ -detector) and concluded that a beta group of end-point energy  $355 \pm 5$  keV would populate the 113.6 keV level of  $^{175}\text{Lu}$ . Bashandy *et al.*<sup>3</sup>, using a medium thick lens spectrometer, measured the relative intensities of the beta groups in the decay of  $^{175}\text{Yb}$  and also the coincidence spectrum of 353 keV beta transition. But no detailed shape analysis of the 353 keV beta transition is available from any one of the above measurements. Hence a detailed shape factor measurement of the inner beta of  $^{175}\text{Yb}$  is undertaken. The experimental shape factor has also been compared with the theoretical predictions in the light of Nilsson model. The validity of CVC theory and the applicability of  $\xi$ -approximation to the beta transition have been discussed in detail.

## 2. EXPERIMENTAL

Ytterbium-175 was obtained from B.A.R.C. as  $\text{YbCl}_3$  in HCl solution. To look for impurities in the source, the singles gamma spectrum of  $^{175}\text{Lu}$  was studied with a 30 c.c. Ge (Li) coaxial type of detector. This showed no detectable impurities. All sources were 2 mm in diameter, on thin mylar foils ( $\approx 250 \mu\text{g}$ /

$\text{cm}^2$ ) and the thickness was found to be less than  $150 \mu\text{g}/\text{cm}^2$ . The 353 keV beta was studied in coincidence with the following 114 keV gamma in Lu-175. The intermediate-image beta ray spectrometer was set to focus the beta spectrum above 200 keV, while the gamma channel was adjusted to accept apart of the 114 keV within a narrow channel width so as to exclude the interference of the 137.6 keV gamma ray. The resolving time of the fast coincidence unit was set up at 24 ns as in the case of  $^{198}\text{Au}$  experiment<sup>6</sup>. All the spectra were roughly scanned in steps of 10 keV in the energy range 200 to 350 keV. For each run, about 900 counts were taken at the maximum of the beta continuum since the intensity of the present beta was very low ( $\approx 3\%$ ). The data were analysed by the methods described elsewhere<sup>6,7</sup>. The experimental shape factor was weighted least square fitted to a shape correction factor of the form  $C(W) = k(1 + aW + cW^2)$ . Figure 1 shows the experimental shape factor curve of the 353 keV beta and the results are summarised in Table I.

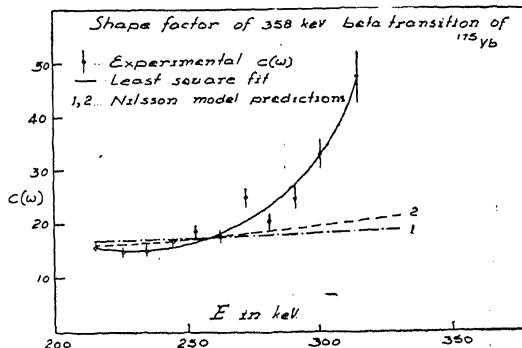


FIG. 1

The value of the end-point energy of the beta transition obtained in the present work is in excellent agreement with those ( $355 \pm 5$  keV) reported by Mize *et al.*<sup>2</sup> and Bashandy *et al.*<sup>3</sup> while it is in disagreement with the value ( $374 \pm 30$ ; 350 keV) reported by Cork *et al.*<sup>1</sup> and De Waard<sup>4</sup>.

TABLE I

| Run No. | $W_0$ (keV) | $C(W) = \frac{1}{2}k(1 + aW + cW^2)$ |                   |
|---------|-------------|--------------------------------------|-------------------|
|         |             | $a(m_0c^2)^{-1}$                     | $c(m_0c^2)^{-2}$  |
| 1       | $358 \pm 2$ | $-1.36 \pm 0.34$                     | $0.468 \pm 0.114$ |
| 2       | $358 \pm 2$ | $-1.36 \pm 0.23$                     | $0.467 \pm 0.079$ |
| 3       | $360 \pm 4$ | $-1.37 \pm 0.43$                     | $0.476 \pm 0.201$ |

## 3. DISCUSSION

The odd-mass nuclide  $^{175}\text{Yb}$  with 105 neutrons lies in the deformed region  $150 \leq A \leq 190$ . Bogdan<sup>8</sup> developed theoretical expressions for both relativistic and non-relativistic nuclear matrix elements, incorporating superfluid model correction<sup>9</sup>. These improved the theoretical log ft values, for the beta transitions of arbitrary forbiddenness, using Nilsson wavefunctions for one particle configuration in a deformed potential. Using these expressions, Bogdan derived the matrix element parameters for  $^{175}\text{Yb}$  beta decay. The ground state of  $^{175}\text{Yb}$  was assigned, using the Nilsson diagram character  $7/2^- (514)$  while the first excited stage of  $^{175}\text{Lu}$  was characterised by  $9/2^+ (404)$ . By taking the deformation parameter  $\delta$  as 0.28, the values of the matrix element parameters for the  $7/2^- \rightarrow 9/2^+$  beta transition ( $\Delta J = 1$ ) are as follows:

$$\begin{aligned} x &= -3.74; & u &= 0; & Z &= 1 \\ w &= 0; & \xi y &= -3.464; & \xi v &= 0. \end{aligned}$$

Berthier and Lipnik<sup>10</sup> considered the beta transition of  $^{175}\text{Yb}$  by assigning the Nilsson orbitals of the initial neutron and of the final proton as  $7/2^- (514)$  and  $9/2^+ (404)$  respectively. By taking the deformation parameter  $\delta = 0.28$ , they expressed the wavefunctions of the initial and final states as:

$$\begin{aligned} \chi_{\Omega=7/2} &= -0.253 | 553 + \rangle + 0.206 | 533 \\ &\quad + \rangle - 0.945 | 554 - \rangle \end{aligned}$$

Final wavefunction:

$$\chi_{\Omega=9/2} = -0.219 | 443 + \rangle + 0.975 | 444 - \rangle.$$

They calculated, the values of the nuclear matrix element starting from the above Nilsson wavefunction as:

$$x = 1, \quad u = -0.511 \quad \text{and} \quad Z = -1.830.$$

In the present analysis, the theoretical shape factor was computed for the above two sets of matrix elements in the exact Simms<sup>11</sup> formalism, treating  $\lambda$  as a free parameter. The experimental shape factor was compared with the theoretical predictions as

shown in Fig. 1. It is evident that the agreement between the experimental shape factor and the theoretical shape factor, following the Nilsson model is somewhat good at low energies, rather than at high energies. It is difficult to comment on the disagreement between the Nilsson model and the experiment in the high energy portion, as the intensity will be very low near the end-point energy due to the poor transition intensity ( $\approx 3\%$ ), resulting in a large statistical spread.

Even though the errors of shape factor coefficients 'a' and 'c' of the present measurement (Table I) are large, due to the low intensity ( $\approx 3\%$ ) of the involved beta, the shape of the  $7/2^- \rightarrow 9/2^+$  beta transition is consistent with the correction term  $C(W) = k(1 + aW + cW^2)$ . The shape deviation observed in the present work is also consistent with the log ft value (7.5) and the large anisotropy reported in the recent beta-gamma correlations<sup>12</sup> and nuclear orientation measurements<sup>13</sup>. These observations suggest that  $\xi$ -approximation is not valid in the case of 353 keV beta transition of  $^{175}\text{Yb}$  even though the value of  $\xi$  (14.99) is much greater than  $W_0 - 1 \approx (0.101)$ , which is generally expected for the breakdown of  $\xi$ -approximation. The value of  $\lambda$  (2.36) obtained is in good agreement with Fujita's estimate<sup>14</sup> thus indicating the validity of 'CVC' theory in the present case.

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# OXIDATION OF INDIGOCARMEIN, ISATIN AND 5-NITRO-ISATIN BY CHLORAMINE-T AND DICHLORAMINE-T

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## ABSTRACT

Indigocarmine undergoes a four electron oxidation per mole with chloramine-T in mineral acids and pH 1 buffer media while aqueous solutions of the compound are oxidized by a solution of dichloramine-T in glacial acetic acid. The products are isatin sulphonate and *p*-toluene sulphonamide and an approximate estimate of the former has been carried out by a spectrophotometric method. 5-Nitro-isatin and isatin can be oxidized by CAT and DCT respectively with a two electron change per mole and volumetric methods have been proposed for their estimation in solution.

## INTRODUCTION

CHLORAMINE-T (CAT) and recently dichloramine-T (DCT) have been successfully employed for estimating a variety of compounds<sup>1,2</sup>. A detailed investigation of the oxidation of indigocarmine and 5-nitro-isatin with CAT has now been carried out. The communication further reports the estimation of indigocarmine and isatin with DCT. Since the oxidation was not instantaneous back titration procedures have been developed.

## MATERIALS AND METHODS

About 2 mM solutions of indigocarmine (E-Merck), isatin (Ward Bleakinsop) and 5-nitro-isatin (Aldrich Chemical Co.) in the appropriate solvents were prepared. Standard buffer systems were employed. Chloramine-T (May and Baker) was purified by the method of Morris *et al.*<sup>3</sup> and its decinormal solution was standardized by the iodometric method. Dichloramine-T was prepared and purified by the method of Jacob and Nair<sup>1</sup> and its decinormal solution in glacial acetic acid was prepared and standardized as above. Beckman DB spectrophotometer was used for optical density measurements.

Table I gives a typical set of results for the oxidation of indigocarmine with CAT in 30 minutes. It is seen from the table that the rate of oxidation is fairly rapid and stoichiometric with a 4-electron change per mole, in mineral acids and pH 1 buffer media. The rate increases with an increase in pH, reaches a maximum of nearly 8-electron change at pH 5 and then decreases. Hence the standard estimation was carried out at pH 1 by adding aliquots of indigo carmine solution in this buffer to a measured excess of 0.1N CAT followed by iodometric back titration of the latter after 30 minutes.

### Oxidation of Indigocarmine with DCT:

Preliminary studies revealed that the rate of oxidation of aqueous indigocarmine solutions with DCT was independent of the dilution. Oxidation beyond the 4-electron change per mole was not noticed, even after 1-2 hours.

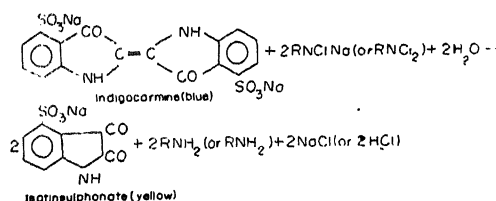
TABLE I

Extent of oxidation of indigocarmine with chloramine-T

| Medium                              | CAT/Indigo | Medium | CAT/I indigo |
|-------------------------------------|------------|--------|--------------|
| 1.0 NH <sub>2</sub> SO <sub>4</sub> | 2.001      | pH 1.0 | 2.007        |
| 0.1 NH <sub>2</sub> SO <sub>4</sub> | 2.005      | pH 2.2 | 2.197        |
| 1.0 NHCl                            | 1.982      | pH 3.0 | 2.273        |
| 0.1 NHCl                            | 2.000      | pH 4.0 | 3.348        |
| 1.0 NHCIO <sub>4</sub>              | 1.888      | pH 5.0 | 3.910        |
|                                     |            | pH 6.0 | 3.318        |
|                                     |            | pH 7.0 | 2.968        |
|                                     |            | pH 8.0 | 1.862        |

Indigocarmine taken = 0.02 m mole ; CAT taken = 1.0 m mole. Time = 30 min. ; Temperature = 26° C. CAT/Indigo = moles of CAT per mole indigocarmine oxidation.

Some typical results of analyses are shown in Table II. The stoichiometry of oxidation of indigocarmine by CAT or DCT could be represented as follows :



Here R = *p*-CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>.

The presence of *p*-toluene sulphonamide (PTS) in the reaction products was detected by paper chromatography<sup>2</sup>. Isatin sulphonate formed had a  $\lambda_{\text{max}}$

TABLE II

Estimation of indigocarmine, 5-nitro-isatin and isatin

| Oxidant CAT   |                |               |            | Oxidant DCT   |            |               |            |
|---------------|----------------|---------------|------------|---------------|------------|---------------|------------|
| Indigocarmine | 5-Nitro-isatin | Indigocarmine | Isatin     | Indigocarmine | Isatin     | Indigocarmine | Isatin     |
| Taken (mg)    | Found (mg)     | Taken (mg)    | Found (mg) | Taken (mg)    | Found (mg) | Taken (mg)    | Found (mg) |
| 7.99          | 8.05           | 3.51          | 3.44       | 4.76          | 4.85       | 3.32          | 3.35       |
| 9.59          | 9.53           | 4.91          | 4.91       | 9.52          | 9.54       | 3.98          | 3.99       |
| 11.99         | 12.07          | 5.61          | 5.73       | 14.27         | 14.31      | 4.65          | 4.62       |
| 13.59         | 13.56          | 7.02          | 7.05       | 16.17         | 16.02      | 4.98          | 4.99       |
| 15.99         | 15.95          | 8.42          | 8.35       | 17.12         | 17.17      | 5.31          | 5.37       |
| 17.51         | 17.51          | 9.83          | 9.83       | 20.93         | 20.99      | 5.98          | 5.99       |
| 19.99         | 19.98          | 10.53         | 10.49      | 23.79         | 23.65      | 6.64          | 6.62       |
| 27.98         | 28.03          | 11.93         | 11.96      | 25.69         | 25.57      | ..            | ..         |
| ..            | ..             | 12.63         | 12.61      | ..            | ..         | ..            | ..         |
| ..            | ..             | 14.04         | 14.10      | ..            | ..         | ..            | ..         |

at 410 nm ( $\log \epsilon = 2.8$ ) and hence was estimated by a spectrophotometric procedure. As pure isatin sulphate was not available, the indigocarmine was oxidised with  $\text{KIO}_3$  to get isatin sulphate<sup>4</sup> for preparing the calibration curve. The results are shown in Table III.

It is interesting to note that the rate of oxidation of indigocarmine by CAT is fastest in the pH range 4-5. This behaviour could probably be attributed to the high rate of disproportionation of monochloramine-T present at this pH to DCT and PTS as suggested by Higuchi *et al.*<sup>5</sup>

**Oxidation of Isatins.**—Extensive investigations showed that definite stoichiometric oxidation corresponding to a 2-electron change per mole could be obtained for aqueous solutions of isatin with DCT and ethanolic solutions of 5-nitro-isatin with CAT.

**Oxidation of isatin with DCT.**—The procedure is similar to the oxidation of indigo carmine with DCT, but required a water content of 30-50% in the reaction mixture and an oxidation period of 75 minutes.

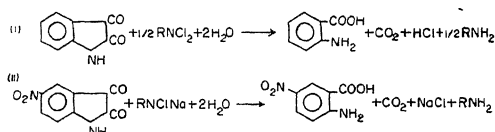
**Oxidation of 5-nitro-isatin with CAT.**—Add aliquots (0.02-0.08 m mole) of a solution of 5-nitro-isatin prepared in 95% ethanol to a known excess ( $\sim 1.0$  m mole) of 0.1 N CAT. Shake the mixture occasionally and after 5 minutes add 10 ml of  $2\text{NH}_4\text{SO}_4$  and 10 ml of 20% KI and titrate against standard thiosulphate. Run a blank with CAT solution alone.

TABLE III

Spectrophotometric estimation of isatin sulphate ( $\lambda_{\text{max}} = 410 \text{ nm}$ )

| Oxidant CAT               |          | Oxidant DCT               |          |
|---------------------------|----------|---------------------------|----------|
| Weight of isatin sulphate |          | Weight of isatin sulphate |          |
| Calculated mg.            | Found mg | Calculated mg             | Found mg |
| 1.54                      | 1.59     | 1.09                      | 1.19     |
| 2.59                      | 2.64     | 3.27                      | 3.44     |
| 4.13                      | 4.01     | 3.82                      | 3.88     |
| 5.17                      | 5.18     | 4.98                      | 5.23     |
| 6.20                      | 6.18     | 5.45                      | 5.68     |
| 10.33                     | 10.31    | 6.54                      | 6.72     |

The stoichiometry of the above oxidations is as follows :



The presence of anthranilic acid ( $R_f = 0.278$ ) in the reaction mixture was detected by paper chromatography, with butanol-ammonia (4:1 V/V) solvent and ethanolic ferric chloride as spray reagent<sup>6</sup>.

Some typical results of analyses are given in Table II. The results are accurate within 0.5%.

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## MONOMINERALIC SYNNEUSIS IN ZIRCON

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### ABSTRACT

Synneusis, which is a diagnostic feature of the fabric of magmatic crystallisation, is strikingly exhibited by zircon. Various habits of zircon aggregates in 'monomineralic synneusis' relation are parallel growths, dumb-bell shaped or necked crystals, synneusis twins, interpenetration twins, overgrowths, fused aggregates of randomly oriented crystals, and odd shapes. Recognition of the various types of zircon in synneusis relation is useful in problems of provenance and petrogenesis.

**Z**IRCON in calc-alkaline rocks and in some of the alkaline rocks is of early crystallisation (Poldervaart<sup>9</sup>, p. 550). These early-formed crystals, while moving about in the melt, come into contact with, and adhere to, growth surfaces of other crystals (Schermerhorn<sup>14</sup>). Such a clustering of the crystals of a mineral occurring in association with other minerals may be termed as "polymineralic synneusis", while the clustering of the crystals made up exclusively of one mineral could be termed as "monomineralic synneusis". Some investigators (Poldervaart<sup>9</sup>, p. 547; Moorehouse<sup>6</sup>; Schermerhorn<sup>14</sup>; Vance<sup>16</sup>, p. 22) have shown the preferential association of zircon with biotite, hornblende, and opaque ores. Instances of the clusters consisting exclusively of zircon crystals have also been cited by Morozewicz<sup>7</sup> (p. 16) and Larsen and Poldervaart<sup>9</sup> (p. 555).

Zircons, isolated from the black sand concentrates, occurring along the east and west coasts of South India and studied by Prasad<sup>12</sup>, were used in the present study. Zircon crystals, in synneusis relation, are classified as: 1. Parallel synneusis; 2. Sub-parallel synneusis; 3. Random synneusis; 4. Synneusis twinning; and 5. Post-synneusis development comprising: (a) Overgrowths; (b) Interpenetration twins; and (c) Odd-shaped crystals.

### PARALLEL SYNNEUSIS

Parallel synneusis in zircon is commonly exhibited by parallel growths in which two or more zircons are joined with their long axes in parallel position (Figs. 1 to 4) and showing essentially parallel extinction. Such types have been referred to as "aggregate crystals" (Poldervaart and Eckelmann<sup>11</sup>). Parallel growth is a case of synneusis in which union of crystals takes place on (100) face. Another case of parallel synneusis is the union of crystals on the pyramidal face (Figs. 19 and 20). Such cases have been interpreted (Jocelyn and Pidgeon<sup>2</sup>) as "cases of central dislocations accompanied by slight displacement".

### DUMB-BELL OR NECKED CRYSTALS

Zircons with a constriction, or a narrow notch in the middle of a grain (Fig. 21), have been referred to as "dumb-bell" or "necked" crystals. In some grains, there may be more than one neck (Fig. 22). According to Murthy *et al.*<sup>8</sup> (p. 35) these types are common in metasomatised and migmatised rocks; while Verspyck<sup>17</sup> (p. 68) and Jocelyn and Pidgeon<sup>2</sup> (p. 593) consider them as resultant of corrosion. But the present writer believes that these necked-crystals are formed by the attachment of two or more well-developed crystals on their basal pinacoidal face (001) just like twinning in the hemimorphic form of a calamine crystal (Dana<sup>1</sup>, p. 182; Fig. 413). But in the case of zircon, (001) is not a twin plane as the plane that is a symmetry plane in the individual crystal cannot become a twin plane. After the attachment of the crystals on (001) face, the grains may be rounded off due either to magmatic corrosion or to abrasion during sedimentary processes. Dana<sup>1</sup> states that cases have been described of the grouping of crystals of the same substance, in which a certain plane is common to the different individuals but which the normal laws of twinning cannot explain. Parallel synneusis in zircon is a case of this type.

Possible mechanisms, proposed by Vance<sup>16</sup> to explain parallel synneusis, involve minimising of interfacial energy by rotation of crystals after contact, or random collision with only those crystals uniting that are in the preferred orientation, or "long-range" forces orienting crystals before contact.

Although synneusis commonly involves union of crystals in parallel position, deviation from such regular grouping, referred to as "sub-parallel synneusis", is not uncommon (Figs. 5, 6 and 7).

Zircon also occurs as fused aggregates (Figs. 8, 9 and 10) which are reported to result from granulation and recrystallisation during ultrametamorphism [Poldervaart and Von Backstrom<sup>10</sup> (p. 467)]. But the present author believes that these fused aggregates are a case of random synneusis (Viola<sup>18</sup>;

Köhler and Raaz<sup>3</sup>; Kraus<sup>4</sup>) in which the participating crystals have erratic orientation (Figs. 8, 9 and 10). The crystals in these cases must have been relatively large at the time of initial contact.

### SYNNEUSIS TWINS

The criteria, suggested by Vance<sup>16</sup>, to distinguish between growth twins (Figs. 16 and 26) and synneusis twins (Figs. 23, 24 and 25) are briefly summarised in Table I.

TABLE I

| Sl. No. | Feature           | Growth Twin   | Synneusis Twin                               |
|---------|-------------------|---|--|
| 1.      | Twinned Crystal   | Only one crystal with a superior fit of the twin units          | Two crystals with a misfit of the twin units |
| 2.      | Twin Units        | Symmetrical   | Asymmetrical                                 |
| 3.      | Composition Plane | Regular, and parallel to the ideal crystallographic orientation | Irregular                                    |

It is significant to note that in some beach placers, the incidence of the frequency of the synneusis twins is more than that of the growth twins in zircon.

### POST-SYNNEUSIS DEVELOPMENT

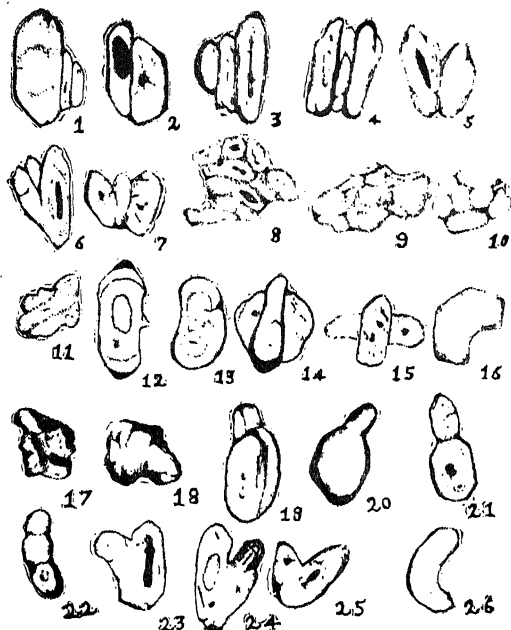
Post-synneusis development in zircon may be incremental or detrimental to the growth of the participating crystals. Post-synneusis developments, incremental to the growth of the crystals, comprise overgrowths and interpenetration twins.

### OVERGROWTHS

In this case, the initial misfit of the crystals in synneusis relation is eliminated by the preferential crystal growth along the re-entrant angles and steps thus eliminating the external morphological irregularities and eventually producing a single crystal (Fig. 12); further growth gives rise to the development of a shell or successive shells. Examination of the overgrowths under the microscope at high magnifications ( $\times 300$ ) reveals the presence of a core consisting of more than one grain (Fig. 13) indicating that the merger is not complete in certain cases.

### INTERPENETRATION TWINS

Interpenetration twins in zircon (Figs. 14 and 15) have also been recorded by Subrahmanyam and Rao<sup>15</sup> (p. 275) and Jocelyn and Pidgeon<sup>2</sup> (Fig. 3:3, p. 590). Synneusis twinning arises through union of two crystals on a crystal face or a combination of faces. The composition surface is thus the planar surface of initial attachment. During continued crystallisation, after synneusis, the two individuals are intergrown as interpenetration twin. Jocelyn and Pidgeon<sup>2</sup> (p. 592) envisage such a form as a combination of synneusis and late growth-twinning.



FIGS. 1-26. Monomineralic synneusis in zircon from the littoral black sand placers of South India. Figs. 1-4. Parallel synneusis on (100) face. Figs. 5, 6, and 7. Sub-parallel synneusis. Figs. 8-11. Fused aggregates in random synneusis. Figs. 12 and 13. Overgrowths. Figs. 14 and 15. Interpenetration twins. Figs. 16 and 26. Geniculate twins. Figs. 17 and 18. Odd-shaped grains. Figs. 19 and 20. Parallel synneusis on (111) face. Figs. 21 and 22. Necked crystals representing parallel synneusis on (001) face. Figs. 23, 24 and 25. Synneusis twins.

### ZIRCONS WITH ODD SHAPES

Odd shapes in zircon are believed to be a result of post-synneusis development, detrimental to the growth of crystals. The boundaries between grains held in synneusis relation are surfaces of weaker bonding than crystallographic planes within single crystals. Hence many of the zircons in synneusis relation might have been detached mechanically

producing odd shapes (Figs. 17 and 18). In necked crystals, the "necks" seem to be weaker than the other surfaces of symneusis relation, as the initial attachment of the grains on (001) is less stable than those of the parallel symneusis on (100) or twin orientations. The surfaces of parallel symneusis with attachment on (111) face are likewise weak mechanically. Paucity of the necked crystals and relatively common occurrence of odd-shaped zircon grains in the placers suggest that they have suffered mechanical breakage in the high energy beach environment.

In view of the undoubted igneous origin of symneusis, recognition of the various types of zircon in symneusis relation is of much value in problems of provenance, parentage, and petrogenesis.

#### ACKNOWLEDGEMENTS

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## EFFECTS OF SEED TREATMENT WITH <sup>60</sup>CO GAMMA RAYS AND MICRONUTRIENTS ON GERMINATION AND GROWTH OF CORN SEEDLINGS

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#### ABSTRACT

Gamma irradiation and soaking in solutions of some micronutrient elements, as presowing treatments of corn seeds, towards improving the germination of seeds, and increasing the growth of seedlings were investigated. The seeds were exposed to 12 irradiation doses of gamma rays ranging from 250–8000 R. It was found that stimulatory effects on the germination percentage and capacity of seeds as well as the height and the dry weight of seedlings were exerted only by the low irradiation doses from 500–1000 R. Soaking cornseeds, before sowing, in any of the 4 concentrations ranging from 250–1000 ppm of molybdenum, manganese and zinc indicated that molybdenum treatment increased plant height and the dry weight of seedlings; 500 ppm molybdenum gave the best effect. Irradiation of 500 ppm molybdenum soaked seeds with low doses of gamma rays stimulated the germination process and early growth of seedlings, with the 500 R dose being most effective.

#### INTRODUCTION

**P**RESOWING treatments of seeds with gamma rays was reported to enhance germination of seeds and to increase the growth of the seedlings (Woodstock and Jüstic, 1967 and Singh, 1970). How-

ever, such stimulatory effects were found to be induced by certain exposure doses which depends upon plant species (Kuzin, 1963).

Soaking of seeds prior to sowing in solutions containing certain micronutrients was also reported



to enhance the germination of seeds and the growth of seedlings (Shkolnik, 1963 and Pavel and Zakova, 1967). Only a few reports were found dealing with the plant responses to the combined seed treatment with gamma rays and micronutrients (Sidarskii, 1963 and Guseva, 1967).

The aim of this work was to investigate the effects of presowing seed treatment, with some trace elements and gamma rays on germination of corn seeds and growth of seedlings.

#### MATERIAL AND METHODS

**Seed Treatments.**—Uniform corn seeds (*Zea mays* cv. Giza hybrid 67) were soaked without aeration, for a 24-hour period in either distilled water or in a solution of ammonium molybdate, zinc sulphate or manganese sulphate. Following the soaking, they were washed thoroughly with distilled water, air-dried and then irradiated, in air, in a  $^{60}\text{Co}$ -gamma cell 220 unit (Atomic Energy Commission, Ottawa, Canada) at a constant exposure rate of 100R/sec. The temperature during irradiation ranged between 28° and 34° C. Thus, there were two experiments, the first one was concerned with the effect of separate seed treatment with either gamma rays or micronutrients. Whereas, the second experiment was concerned with the combination treatments which were limited to 500, 1000 and 2000 R with 500 ppm Mo only.

**Cultivation of Plants.**—Treated seeds were immediately planted in sand, at a rate of 10 per each betumin-coated earthenware pots No. 25. A half strength complete nutrient solution of Hoagland and Arnon (1941) was utilized for duration of the experimental. Pots were maintained under the prevailing climatic conditions at the Experimental Station of the National Research Centre at Dokki, Giza. Each treatment was replicated six times, and each replicate as represented by one pot.

**Germination Studies.**—Germination measurements were carried out on seeds, 200 per treatment, allowed to germinate on moistened cotton wool in 25 cm. glass evaporating dishes. Each treatment had 4 replicates. Germinated seeds (with emergent plumules) were counted daily and the results are reported (Table I) as the following values; the germination percentage after 5 and 14 days, and the germination rate index which was calculated from the following formula.

$$\frac{a + (a + b) + (a + b + c) + \dots}{n(a + b + c + \dots)}$$

where  $a$ ,  $b$  and  $c$  are counts of seeds germinated after the first, second and third day respectively, and  $n$  is the number of counts.

**Growth Measurements.**—Stem height and dry weight of shoots and roots were determined for 14-days old

seedlings. Samples were dried at 105° C to a constant weight and cooled down in a vacuum desiccator.

#### RESULTS

##### Determination of the Effective Range of Radiation

Results of exposing seeds, soaked in distilled water, to any of the 12 doses ranging from 250 to 8000 R are shown in Table I. In general, germination was stimulated by 500 and 750 R only; other doses were ineffective or inhibitory. There was no significant difference in the germination rate index due to different exposure doses.

TABLE I

Effects of irradiation with various exposure doses on germination of corn seeds

| Sl. No.       | Irradiation dose, R | Germination percentage |               | Germination rate index |
|---------------|---------------------|------------------------|---------------|------------------------|
|               |                     | After 5 days           | After 14 days |                        |
| 1.            | 0                   | 90.0                   | 93.3          | 0.48                   |
| 2.            | 250                 | 93.3                   | 93.3          | 0.49                   |
| 3.            | 500                 | 96.6                   | 100.0         | 0.49                   |
| 4.            | 750                 | 93.3                   | 100.0         | 0.49                   |
| 5.            | 1000                | 93.6                   | 96.6          | 0.49                   |
| 6.            | 1500                | 93.3                   | 93.3          | 0.49                   |
| 7.            | 2000                | 93.3                   | 96.6          | 0.46                   |
| 8.            | 3000                | 80.0                   | 93.6          | 0.46                   |
| 9.            | 4000                | 80.0                   | 90.0          | 0.45                   |
| 10.           | 5000                | 60.0                   | 67.0          | 0.45                   |
| 11.           | 6000                | 50.0                   | 66.0          | 0.48                   |
| 12.           | 7000                | 50.0                   | 50.0          | 0.45                   |
| 13.           | 8000                | 36.6                   | 36.6          | 0.45                   |
| L.S.D., at 5% |                     | 3.58                   | 3.76          | N.S.                   |

Similar but not identical results were obtained for the stem height and dry weight of 14 days old seedlings grown from the irradiated seeds (Table II). Again here the low irradiation doses, particularly the 500, 750 and 1000 R doses, were effective in stimulating

TABLE II  
 Average values of height, and dry weight of corn seedlings developed from seeds irradiated with various exposure doses

| Sl. No.      | Irradiation dose, R | Seedling height, cm | Dry weight, g/100 seedlings |      |                | Shoot/root ratio (On dry wt. basis) |
|--------------|---------------------|---------------------|-----------------------------|------|----------------|-------------------------------------|
|              |                     |                     | Shoot                       | Root | Whole seedling |                                     |
| 1.           | 0                   | 26.30               | 1.81                        | 3.16 | 5.97           | 0.57                                |
| 2.           | 250                 | 25.80               | 1.92                        | 3.04 | 4.96           | 0.63                                |
| 3.           | 500                 | 33.90               | 2.75                        | 4.89 | 7.64           | 0.56                                |
| 4.           | 750                 | 33.56               | 2.58                        | 4.67 | 7.25           | 0.55                                |
| 5.           | 1000                | 39.16               | 2.82                        | 4.56 | 7.38           | 0.61                                |
| 6.           | 1500                | 29.40               | 2.21                        | 4.61 | 6.82           | 0.48                                |
| 7.           | 2000                | 24.56               | 2.11                        | 3.85 | 5.96           | 0.55                                |
| 8.           | 3000                | 30.46               | 2.06                        | 3.58 | 5.64           | 0.58                                |
| 9.           | 4000                | 27.16               | 2.10                        | 3.26 | 5.36           | 0.64                                |
| 10.          | 5000                | 26.56               | 2.32                        | 2.89 | 5.21           | 0.80                                |
| 11.          | 6000                | 26.86               | 1.09                        | 2.78 | 4.67           | 0.68                                |
| 12.          | 7000                | 26.40               | 1.55                        | 2.52 | 4.07           | 0.62                                |
| 13.          | 8000                | 23.05               | 1.58                        | 1.92 | 3.56           | 0.82                                |
| L.S.D. at 5% |                     |                     | 3.22                        | 0.62 | 1.28           | 2.06                                |

growth. As revealed by calculating the shoot/root ratio, the irradiation effect was equally well on both parts of the developing seedlings.

#### *Determination of the Effective Type and Concentration of Micronutrients:*

Results of soaking seeds in any of 4 concentrations, viz., 250, 500, 750 and 1000 ppm of solutions of each of the microelements are presented in Table III. All treatments generally resulted in a decreased growth measured as length of seedlings except the 500 ppm molybdenum treatment in which case an increase in height as well as in the dry weight of whole seedlings was evident. It is of interest that the dry weight increase resided totally in the root organ. The increase in dry weight or at least absence of inhibition of the roots was, however, a constant feature

of all treatments with the three microelements. (Table III).

#### *Combined Effect of Irradiation and Molybdenum:- Soaking*

Results of irradiating molybdenum soaked seeds with any of three doses, namely, 500, 1000, and 2000 R on germination and growth of 14 days old seedlings are shown in Tables IV and V respectively. These results substantiated the above-mentioned ones in as much as irradiation increased the germination percentage of treated seeds with the 500 R dose being more effective. Molybdenum soaking was rather slightly inhibiting in this regard.

As shown in Table V irradiation with 500 or 1000 R but not with 2000 R tended generally to increase the seedling height. This stimulatory effect was more

TABLE III

*Average values of height, and dry weight of corn seedlings developed from the seeds treated with various concentrations of micronutrient elements*

| Type and concentration of element, ppm | Seedling height, cm. | Dry weight, g/100 seedlings |      |                | Shoot/Root ratio (On dry wt. basis) |
|--|----------------------|-----------------------------|------|----------------|-------------------------------------|
|  |                      | Shoot                       | Root | Whole seedling |                                     |
| Water                                  | 26.30                | 1.81                        | 3.16 | 4.97           | 0.57                                |
| Mo                                     | 250                  | 1.81                        | 4.62 | 6.43           | 0.39                                |
|  | 500                  | 1.99                        | 5.05 | 7.04           | 0.39                                |
|  | 750                  | 1.46                        | 4.62 | 6.08           | 0.32                                |
|  | 1000                 | 1.09                        | 4.89 | 5.98           | 0.22                                |
| Mn                                     | 250                  | 1.08                        | 3.10 | 4.18           | 0.34                                |
|  | 500                  | 1.30                        | 3.85 | 5.15           | 0.34                                |
|  | 750                  | 1.36                        | 3.45 | 4.81           | 0.39                                |
|  | 1000                 | 1.28                        | 3.60 | 4.88           | 0.36                                |
| Zn                                     | 250                  | 1.26                        | 3.47 | 4.73           | 0.36                                |
|  | 500                  | 1.28                        | 3.41 | 4.69           | 0.37                                |
|  | 750                  | 1.18                        | 3.28 | 4.46           | 0.36                                |
|  | 1000                 | 1.03                        | 3.77 | 4.80           | 0.27                                |
| L.S.D. at 5%                           | 2.45                 | N.S.                        | 1.26 | 1.42           | ..                                  |

marked in the seedlings developed from irradiated, molybdenum soaked seeds. Molybdenum soaking in itself, increased the seedling height more markedly than did any of the irradiation treatments of water soaked seeds. The effect of irradiating water or molybdenum soaked seeds on the dry weight of the seedlings shoots paralleled more or less its effects on their height. Although the dry weight of root of seedlings grown from water soaked seeds was affected in much the same way as did their shoots, that of seedlings developed from molybdenum soaked seeds responded rather differently. In the latter case an inverse relationship existed between the exposure dose and the dry weight of the roots. The molybdenum treatment, on its own, induced an increase of a slightly more than 65% in the dry weight of roots, but had no effect whatsoever on the shoot's dry weight.

Of all experimental treatments, irradiating molybdenum soaked seeds with 500 R was most effective in improving the growth of the seedlings.

#### DISCUSSION

The enhancement of seed germination process by gamma irradiation may be attributed to its known effect on activation of respiratory enzymes particularly those associated with mitochondria (Woodstock and Combs, 1965 and Singh, 1970). This would lead to a correspondingly increased rate of conversion of respiratory substrates to smaller molecules or building blocks required for synthesis of new cell constituents (Toole *et al.*, 1956). Moreover, the irradiation-increased enzyme activity may lead to an increased formation of indole auxins or of their precursors, thus, stimulating subsequent plant growth (Kutacek *et al.*, 1966). Gamma irradiation-induced stimu-

TABLE IV  
*Effects of irradiation and/or molybdenum soaking on germination of corn seeds*

| Irradiation dose, R | Germination percentage |      |               |       |       |      | Germination rate index |
|---------------------|------------------------|------|---------------|-------|-------|------|------------------------|
|                     | After 5 days           |      | After 14 days |       |       |      |                        |
|                     | Water                  | Mo   | Water         | Mo    | Water | Mo   |                        |
| 0                   | 90.0                   | 89.0 | 93.3          | 99.0  | 0.48  | 0.51 |                        |
| 500                 | 96.6                   | 91.0 | 100.0         | 100.0 | 0.49  | 0.52 |                        |
| 1000                | 93.6                   | 99.0 | 96.6          | 100.0 | 0.49  | 0.52 |                        |
| 2000                | 93.3                   | 90.0 | 96.6          | 88.3  | 0.46  | 0.52 |                        |

| L.S.D. at 5%    | G. percentage | G. capacity | G.R. index |
|-----------------|---------------|-------------|------------|
| For irradiation | 1.03          | 0.51        | N.S.       |
| For soaking     | N.S.          | 0.99        | N.S.       |
| For interaction | N.S.          | 1.014       | N.S.       |

lation of plant growth has been reported for various plant species including corn, wheat, radish, sugar beet, etc. (Wassil et al. 1961; Kaur, 1963; Sparrow, 1966; and Woodcock and Lister, 1967).

In a more or less linear manner, the increased molybdenum content of seeds, subsequent to their soaking, is supposed to act through enhancement of the nitrate reductase (Pate and Zinselmeier, 1971) and nitrilase (Pate and Zinselmeier, 1971) enzyme systems. This would lead to enhancement of plant nitrogen and carbohydrate metabolism and, as a consequence, to accumulation of dry matter, particularly in the roots where nitrate is absorbed.

Irradiation of Moberated seeds with 500 R gamma rays gave the most favourable effect on seed germination and the growth of the developed seedlings, which may indicate that these two factors had an additive effect.

The increased rate of dry matter accumulation of seedlings may be taken as a good indication for acceleration of subsequent plant growth and may provide a basis for grading the untreated plants.

#### ACKNOWLEDGMENT

The authors are greatly indebted to the Egyptian Atomic Energy Establishment of Egypt for offering the facilities of seed irradiation with gamma rays.

TABLE V  
*Effects of irradiation and/or soaking in ammonium molybdate on growth of the developed seedlings as represented by the height and dry weight*

| Irradiation dose, R | Seedling height:<br>cm |      | Dry weight, g 100 seedlings |       |             |      |                |      | Shoot: Root ratio<br>on dry wt. basis |      |
|---------------------|------------------------|------|-----------------------------|-------|-------------|------|----------------|------|---------------------------------------|------|
|                     |                        |      | Shoot                       |       | Root        |      | Whole seedling |      |                                       |      |
|                     | Water                  | Mo   | Water                       | Mo    | Water       | Mo   | Water          | Mo   | Water                                 | Mo   |
| 0                   | 26.3                   | 33.0 | 1.81                        | 1.82  | 3.16        | 5.14 | 4.97           | 6.96 | 0.57                                  | 0.35 |
| 500                 | 33.9                   | 43.5 | 2.75                        | 3.90  | 4.89        | 4.69 | 7.67           | 8.59 | 0.56                                  | 0.78 |
| 1000                | 32.2                   | 44.1 | 2.82                        | 3.71  | 4.56        | 3.83 | 7.38           | 7.54 | 0.61                                  | 0.97 |
| 2000                | 27.2                   | 39.0 | 2.11                        | 2.25  | 3.85        | 3.92 | 5.96           | 6.17 | 0.55                                  | 0.58 |
| L.S.D. at 5% :      | Plant height           |      | Shoot                       | Root  | Whole plant |      |                |      |                                       |      |
| For irradiation     | 1.30                   |      | 0.120                       | 0.035 | 0.54        |      |                |      |                                       |      |
| For soaking         | 2.59                   |      | 0.228                       | 0.165 | 0.33        |      |                |      |                                       |      |
| For interaction     | N.S.                   |      | N.S.                        | 0.168 | N.S.        |      |                |      |                                       |      |

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## LETTERS TO THE EDITOR

### SENSITIZED EMISSION SPECTRA OF 1, 4-DIHYDROXY, 2-METHYL ANTHRAQUINONE

#### Introduction

JAYSWAL AND SINGH<sup>1-3</sup> have recorded the sensitized emission spectra of *p*-benzoquinone and its derivatives in vapour phase and the transition involved in the emission has been shown to be triplet-singlet type. This assignment was recently supported by the phosphorescence spectrum of *p*-benzoquinone, recorded by Briegleb *et al.*<sup>4</sup> The method of Jayswal *et al.* has been extended for investigating the emission spectra of many condensed ring quinones<sup>5-7</sup>. The emission spectrum of quinizarin in vapour and in solution has already been investigated by Baruah *et al.*<sup>5</sup> and also by Shigorin<sup>6</sup>. It has been seen that the assignment of the electronic transition, in case of quinizarin and similar molecules, was ambiguous. As a matter of fact, the emission spectra of quinizarin<sup>5</sup> and 1, 4, 5, 8-tetrahydroxy anthraquinone vapour<sup>6</sup> were believed to be of  $n-\pi^*$  type, although the solution spectral data showed that the transition is actually a  $\pi-\pi^*$  one.

On structural grounds, one might expect the electronic states of 1, 4-dihydroxy, 2-methylanthraquinone to resemble those of quinizarin. The present work is undertaken with a view to draw some definite conclusion about the nature of the electronic transition and multiplicities of the states (whether  $n-\pi^*$  or  $\pi-\pi^*$  and T-S or S-S) involved in the emission spectra of these molecules. In vapour phase one cannot employ the usual techniques like disappearance of  $n-\pi^*$  transition in acid media and comparative oscillator strengths to identify the type of electronic transition, whether  $n-\pi^*$  or  $\pi-\pi^*$ . However, the substitution effect, in such cases goes a long way for such identification. Several substituted quinizarins have been tried but only the molecule 1, 4-dihydroxy, 2-methyl anthraquinone could be successfully excited, using the method of sensitization.

#### Experimental Procedure

The emission spectrum of the molecule was recorded with the help of an uncondensed transformer discharge using a  $\pi$ -type discharge tube. The optimum condition for the luminescence glow was obtained by gradually heating the tube uniformly. The faint yellowish emission glow was recorded on a Fuess Glass Spectrographs using Kodak HP-3 plate, with a slit width of 70  $\mu$  and exposure time of about twenty hours.

#### Results and Discussion

The molecular symmetry for 1, 4-dihydroxy, 2-methyl anthraquinone is taken as  $C_s$  ( $C_{2v}$  for quinizarin). The

emission spectrum of 1, 4-dihydroxy, 2-methyl anthraquinone lies in the region 5.0-5.5  $\times 10^4$  Å and consists of about twelve weak and diffuse bands. The first band at 19743  $\text{cm}^{-1}$  of the short wavelength side has been identified as the 0, 0 band of the system. The analysis of the spectrum can conveniently be made with two ground state fundamentals of frequencies 397 and 970  $\text{cm}^{-1}$ , the former corresponding to ring vibration (388  $\text{cm}^{-1}$  in anthraquinone, 348  $\text{cm}^{-1}$  in quinizarin and 390  $\text{cm}^{-1}$  in anthracene). This mode of vibration has been observed as a very strong band in the emission spectrum of all quinone derivatives. The frequency, 970  $\text{cm}^{-1}$ , may also correspond to a ring vibration (skeletal mode in-plane) having its counterpart in the infrared spectrum at 956  $\text{cm}^{-1}$ . It is worthwhile to note that no bands corresponding to the fundamental frequencies of carbonyl stretching mode have been observed. This indicates that the transition does not involve the excitation of the loosely bound  $\pi$ -electrons ( $n-\pi^*$  transition). In the earlier work, on the emission spectrum of quinizarin<sup>5</sup> (1, 4-dihydroxy anthraquinone), a very strong band with frequency 1680  $\text{cm}^{-1}$  was assigned to C=O stretching mode. The interpretation seems to be ambiguous on the basis that the transition was assumed to be of  $\pi-\pi^*$  type. An alternate way of assigning 1680  $\text{cm}^{-1}$  is to interpret it as double quanta of 840  $\text{cm}^{-1}$ , which probably corresponds to 970  $\text{cm}^{-1}$  in the present work.

The ring vibration gives rise to strong bands in the emission spectra of all the quinone derivatives. Table I gives the values for this mode of vibration for a number of quinone derivatives. It is observed that the magnitude of this mode remains practically unaltered in all such cases.

TABLE I

Ring bending mode observed in the emission spectrum of quinone derivatives

| Sl. No. | Compound  | Frequency $\text{cm}^{-1}$ |
|---------|---|----------------------------|
| 1.      | Anthraquinone   | 388                        |
| 2.      | 2-hydroxy anthraquinone                               | 380                        |
| 3.      | 1, 4-dihydroxy anthraquinone                          | 398                        |
| 4.      | 2-chloro anthraquinone                                | 380                        |
| 5.      | 2-methyl anthraquinone                                | 377                        |
| 6.      | 1-methylamino anthraquinone                           | 398                        |
| 7.      | 1, 4-dihydroxy, 2-methyl anthraquinone (Present work) | 397                        |

The emission spectrum of 1, 4-dihydroxy, 2-methyl anthraquinone is very much similar to that of quinizarin except for the shift of the electronic origin in the former towards longer wavelength side by  $244\text{ cm}^{-1}$ , which is apparently due to methyl group substitution at 2-position. This shows that the present emission spectrum has the characteristic of a  $\pi\text{-}\pi^*$  transition.

TABLE II

Vibrational analysis of the  $\pi\text{-}\pi^*$  emission spectrum of  
1, 4-dihydroxy, 2-methyl anthraquinone

| Sl. No. | Frequency $\text{cm}^{-1}$ | Separation from (0, 0), $0.0\text{ cm}^{-1}$ | Interpretation |
|---------|----------------------------|--|----------------|
| 1.      | 16859                      | 2884   | 0-3 970        |
| 2.      | 17074                      | 2649   |                |
| 3.      | 17459                      | 2284   |                |
| 4.      | 17658                      | 2085   |                |
| 5.      | 17803                      | 1940   |                |
| 6.      | 17886                      | 1857   | 0-2 970        |
| 7.      | 18346                      | 1397   | 0-397-970      |
| 8.      | 18554                      | 1189   | 0-3 397        |
| 9.      | 18773                      | 970  | 0-970          |
| 10.     | 18957                      | 786  | 0-2 397        |
| 11.     | 19346                      | 397  | 0-397          |
| 12.     | 19743                      | 0  | (0, 0)         |

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#### ON THE FRANCK-CONDON FACTORS AND r-CENTROIDS OF ( $A^1\Sigma-X^1\Sigma$ ) PtO AND SiF ( $D^2\Sigma-X^2\Sigma$ ) MOLECULES

THE astral character of SiF and PtO molecules has been established from the occurrence of the bands of these molecules in the radiations of  $\beta$ -pegasi star, sunspots, etc.<sup>1</sup>. As such from the theoretical deter-

minations of intensities, i.e., Franck-Condon (F-C) factors and  $r$ -centroids, one could infer some of the physical conditions prevailing in the distant stars like  $\beta$ -pegasi. Hence an attempt towards the computation of F-C factors and  $r$ -centroids has been carried out in this paper.

Intensity of a band due to the electron jump from  $V'$  level of one electronic state to  $V''$  level of another electronic state is given by

$$I_{V', V''} = \frac{64\pi^4 CN_{V'} \gamma^4}{3} \left[ \int \psi_{V'} R_e(r) \psi_{V''} dr \right]^2$$

Here

$C$  = Velocity of light

$N_{V'}$  = molecular population in the excited state

$\gamma$  = frequency of transition

$R_e(r)$  = electronic transition moment

$r$  = internuclear distance.

The square of the bracketed quantity is known as the relative transition probability ( $q_{V', V''}$ ) between two vibrational levels  $V'$  and  $V''$ . If one assumes  $R_e(r)$  as slowly varying function of  $r$  then it may be replaced by an average value  $R_e$ . Thus  $q_{V', V''}$  may be put in the form

$$q_{V', V''} = R_e^{-2} \left[ \int \psi_{V'} \psi_{V''} dr \right]^2$$

The quantity  $\int \psi_{V'} \psi_{V''} dr$  is called overlap integral and its square is known as F-C factor.

F-C factors may be computed from R-K-R or R-K-R- $V^{2-3}$  potential energy curve or by the method proposed by Fraser and Jarman<sup>6</sup>. To avoid the tiresome calculations, we have utilised the latter method with  $r_e$ -shift corrections and they are presented in the 1st row of Tables II and III, for PtO and SiF molecules respectively. The molecular parameters<sup>7</sup> used in the calculations are collected in Table I.

TABLE I

Molecular parameters of PtO and SiF molecules

| Mole-<br>cules | Mole-<br>cular<br>state | We<br>( $\text{cm}^{-1}$ ) | WeXe<br>( $\text{cm}^{-1}$ ) | Be<br>( $\text{cm}^{-1}$ ) | $\alpha \cdot 10^{-3}$<br>( $\text{cm}^{-1}$ ) | $r_e$<br>( $\text{\AA}$ ) |
|----------------|-------------------------|----------------------------|------------------------------|----------------------------|--|---------------------------|
| PtO            | $A^1\Sigma$             | 727.07                     | 5.42                         | 0.35385                    | 2.91   | ..                        |
|                | $X^1\Sigma$             | 851.09                     | 4.97                         | 0.38223                    | 2.83   | 1.727                     |
| SiF            | $D^2\Sigma$             | 1003.2                     | 5.64                         | 0.625                      | 5.0  | 1.54                      |
|                | $X^2\Sigma$             | 857.20                     | 4.74                         | 0.58138                    | 4.90   | 1.6003                    |

TABLE II

*F-C Factors and r-centroids for  $A^1\Sigma-X^1\Sigma$  system of PtO molecule*

| $V' V''$ | 0                                  | 1                                  | 2                                  | 3                                  | 4                        | 5                        |
|----------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|--------------------------|--------------------------|
| 0        | 0.4254<br>1.764<br>1.763<br>5902.3 | 0.3880<br>1.810<br>1.810<br>6210.6 | 0.1499<br>1.860<br>1.859<br>6548.5 | 0.0321<br>1.915<br>1.913           | 0.0042<br>1.976<br>1.972 | 0.0003<br>2.044<br>2.043 |
| 1        | 0.3358<br>1.727<br>1.726<br>5663.0 | 0.0036<br>1.770<br>1.770           | 0.2838<br>1.817<br>1.816<br>6255.0 | 0.2614<br>1.867<br>1.865<br>6594.0 | 0.0316<br>1.922<br>1.920 | 0.0169<br>1.983<br>1.977 |
| 2        | 0.1567<br>1.694<br>1.694<br>5445.6 | 0.1902<br>1.734<br>1.734<br>5707.0 | 0.0530<br>1.777<br>1.778           | 0.1102<br>1.823<br>1.822           | 0.2845<br>1.874<br>1.872 | 0.1592<br>1.929<br>1.926 |
| 3        | 0.0570<br>1.662<br>1.660<br>5248.0 | 0.2074<br>1.700<br>1.700<br>5489.0 | 0.0419<br>1.741<br>1.741           | 0.1423<br>1.784<br>1.785           | 0.0135<br>1.830<br>1.830 | 0.2356<br>1.881<br>1.880 |
| 4        | 0.0180<br>1.633<br>1.635           | 0.1221<br>1.669<br>1.666           | 0.1559<br>1.707<br>1.707           | 0.0002<br>1.747<br>1.748           | 0.1598<br>1.791<br>1.792 | 0.0053<br>1.837<br>1.837 |
| 5        | 0.0052<br>1.606<br>1.609           | 0.0538<br>1.640<br>1.641           | 0.1515<br>1.676<br>1.676           | 0.0733<br>1.714<br>1.715           | 0.0330<br>1.754<br>1.756 | 0.1159<br>1.797<br>1.799 |

The  $r$ -centroid of a transition  $V' \rightarrow V''$  which is defined as

$$\bar{r}_{V', V''} = \frac{\int_0^\infty \psi_{V'} r \psi_{V''} dr}{\int_0^\infty \psi_{V'} \psi_{V''} dr}$$

has been determined by both quadratic equation and graphical methods developed by Nicholls and Jarman 1956<sup>8</sup>, and entered in 2nd and 3rd rows of Tables II and III. 4th row in Table II gives the wavelengths of the corresponding bands in Å.

A study of Table II reveals that there is a termination of  $V'$  progression corresponding to  $V'' = 0$  at  $V' = 3$  and  $V''$  progression corresponding to  $V' = 0$  at  $V'' = 2$  which is justified by experimental observations. The absence of 1, 1 and 2, 2 bands may be attributed to the low value of F-C factors but

3, 3; 4, 4 and 5, 5 bands possess considerable magnitude of F-C factors. Hence these bands ought to be observable experimentally. Besides, it should be possible to observe bands such as 2, 4; 2, 5; 3, 5; 4, 2 and 5, 2 as their F-C factors are comparable with F-C factors of those which have been observed.

From Table III, depending upon the F-C factors, it may be very easily concluded that 0.0 band is the most intense in  $D^2\Sigma^- - X^2\pi$  system of SiF molecule. Further bands in  $V''$  progression corresponding to  $V' = 0$  after  $V'' = 1$  and  $V'$  progression corresponding to  $V'' = 0$  after  $V' = 1$  cannot be observed. But bands such as 1, 2; 2, 1; 3, 2 and 5, 3 seem to be more probable.

$r$ -Centroids in Table II increase with increasing wavelengths which is a sign of red shaded system. In case of  $D^2\Sigma^- - X^2\pi$  system of SiF, the quantity  $r_{e'} + r_{e''}/2 \approx \bar{r}_{0,0}$ , hence it may be concluded that



TABLE III  
F-C factors and r-centroids for  $D^2\Sigma^+-X^2\pi$  system of SiF molecule

| V' V" | 0                        | 1                        | 2                        | 3                        | 4                        | 5                        |
|-------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 0     | 0.5577<br>1.573<br>1.574 | 0.3123<br>1.525<br>1.524 | 0.0999<br>1.478<br>1.476 | 0.0241<br>1.432<br>1.426 | 0.0049<br>1.385<br>1.365 | 0.0009<br>1.336<br>1.330 |
| 1     | 0.3357<br>1.630<br>1.631 | 0.0939<br>1.581<br>1.582 | 0.2948<br>1.534<br>1.533 | 0.1842<br>1.488<br>1.485 | 0.0674<br>1.442<br>1.436 | 0.0185<br>1.396<br>1.380 |
| 2     | 0.0906<br>1.689<br>1.691 | 0.3517<br>1.638<br>1.639 | 0.0001<br>1.590<br>1.590 | 0.1742<br>1.543<br>1.541 | 0.2128<br>1.497<br>1.494 | 0.1139<br>1.452<br>1.456 |
| 3     | 0.0144<br>1.751<br>1.758 | 0.1890<br>1.697<br>1.700 | 0.2429<br>1.646<br>1.647 | 0.0496<br>1.598<br>1.598 | 0.0647<br>1.552<br>1.550 | 0.1884<br>1.506<br>1.504 |
| 4     | 0.0015<br>1.816<br>1.826 | 0.0458<br>1.758<br>1.766 | 0.2536<br>1.705<br>1.708 | 0.1200<br>1.654<br>1.655 | 0.1196<br>1.606<br>1.606 | 0.0079<br>1.560<br>1.559 |
| 5     | 0.0001<br>1.885<br>1.898 | 0.0064<br>1.823<br>1.833 | 0.0899<br>1.766<br>1.774 | 0.2713<br>1.713<br>1.715 | 0.0350<br>1.662<br>1.662 | 0.1573<br>1.615<br>1.614 |

the potential energy curves are not very anharmonic.

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#### PARTICLE SIZE DISTRIBUTION OF NONAQUEOUS EMULSIONS

THE existence of two distinct emulsion types, *i.e.*, either an oil-in-water (O/W) or water-in-oil (W/O) emulsion, was first pointed out by Ostwald<sup>1</sup>. It is really surprising that no attention has been paid so far, to the chemistry of a new possible third type of emulsion, which may be called as oil-in-oil (O/O) emulsion. In the present work, particle size distribution of nonaqueous emulsion, with benzene and ethylene glycol as two nonaqueous disperse and continuous phases and sodium dioctylsulphosuccinate (anionic surfactant) as emulsifier, has been studied.

In dispersed systems, according to Gaussian or normal distribution law, the logarithmic size frequency distribution curve is defined by<sup>2</sup>

$$G(D) = \frac{\Sigma n}{2.303 \log \sigma g \sqrt{2\pi}} \exp \left[ -\frac{(\log D - \log D_g)^2}{2 \log^2 \sigma g} \right] \quad (1)$$

where  $G(D)$  is the probability or frequency of observation of particle diameter  $D$ ,  $n$  is the number of particles within size interval  $\Delta D$ ,  $D_g$  is the geometric mean diameter of particles and  $\sigma g$  is the

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geometric standard deviation. The last two parameters are determined by the expressions

$$\log Dg = \frac{\sum n \log D}{\sum n} \quad (2)$$

and

$$\log \sigma g = \sqrt{\frac{\sum [n(\log D - \log Dg)^2]}{\sum n}} \quad (3)$$

Another parameter, generally desired for the emulsion systems, is the mean volume-surface diameter ( $D_{vs}$ ) of the droplets, given by

$$D_{vs} = \frac{\sum n D^3}{\sum n D^2} \quad (4)$$

One more useful term is the particle size  $D_{mf}$  about which is clustered those particles with the greatest frequency of occurrence or, more precisely, for which the particle density  $n/\Delta D$  is maximum and is defined by<sup>3</sup>

$$\log D_{mf} = \log Dg - 2.303 \log^2 \sigma g$$

The nonaqueous emulsion was prepared with equal volumes of benzene and ethylene glycol and 2.5% concentration of sodium dioctylsulphosuccinate (w/v% of ethylene glycol). The heterogeneous mixture of benzene and ethylene glycol containing surfactant was homogenized with a Braun emulsator.

After homogenizing, the emulsion type was determined by dye solubility method<sup>4</sup> and the emulsion was found to be benzene in ethylene glycol type.

Particle size distribution of oil-in-oil emulsion was determined by photomicrographic method<sup>5</sup>, by taking photomicrographs of microscopic slide of emulsion on ORWO, NP 27, 400 ASA cut film with Carl Zeiss Jena microscope equipped with attachment camera using 25 × projection system and 40 × objective.

The values of  $G(D)$ ,  $Dg$ ,  $\sigma g$ ,  $D_{vs}$  and  $D_{mf}$  of the emulsion were calculated from Eqs. (1) through (5). The values of these parameters for the particular system are  $Dg = 2.853 \mu$ ,  $\sigma g = 1.607 \mu$ ,  $D_{vs} = 4.585 \mu$ , and  $D_{mf} = 2.278 \mu$ .

The size-frequency distribution of the nonaqueous emulsion can be explained with a knowledge of particle density, i.e., the extent to which particles are grouped by count about some given diameter. The most convenient expression for particle density is the number of particles per unit size interval,  $n/\Delta D$ . By the simple expedient of division by  $\Delta D$ , all  $n$  values are normalized to an interval of 1 micron. The plot of  $n/\Delta D_{vs}$ , the mid-interval diameter  $D$ , is shown in Fig. 1. The curve reaches its peak value at the particle diameter of greatest frequency of occurrence  $D_{mf}$ .

If we plot particle density  $n/\Delta D$  against the logarithm of the size interval  $D$ , the curve becomes symmetrical as shown in Fig. 2, and its peak value remains the same as earlier. If, we take each point on this curve and weight it according to the corresponding particle size  $D$ , the resulting values of  $nD/\Delta D$  yield the dotted curve of Fig. 2, which is also symmetrical but reaches its peak at the number median diameter  $Dg$  of 2.853  $\mu$ .

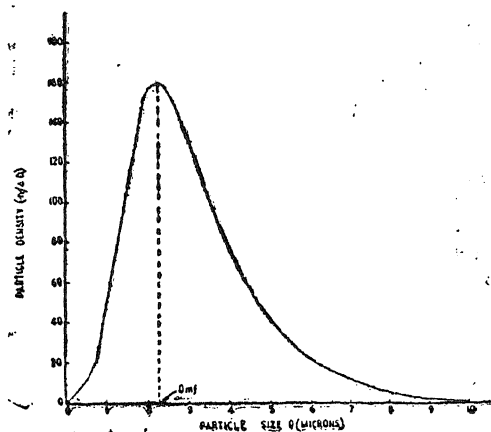


FIG. 1. Particle density vs. size.

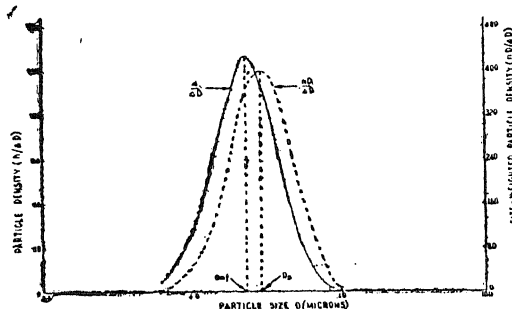


FIG. 2. Particle density and size-weighted particle density vs. size.

The physical significance of the frequency function  $G(D)$  is that it is numerically equal to  $nD/\Delta D$  and it has actually been found that each calculated  $G(D)$  value from Eq. (1) of the emulsion is approximately equal to its corresponding  $nD/\Delta D$  value. The dotted curve of Fig. 2 is effectively a plot of  $G(D)$  vs.  $\log D$ . This curve graphically illustrates that the droplets are symmetrically grouped in some coherent manner with respect to the logarithms of their diameters, the axis of symmetry being the logarithm of the number geometric diameter  $Dg$ . The curves of Fig. 2 thus

illustrate a graphical method for determining  $D_g$  and  $D_{mf}$  of nonaqueous emulsions.

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May 9, 1975.

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### COMPLEXATION BETWEEN DIAZEPAM AND OTHER CHEMICAL AGENTS

DIAZEPAM, 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one, is a psychotherapeutic agent, synthesized by Sternbach and Reeder<sup>1</sup>. The drug exhibits a low water solubility of  $50 \mu\text{g/ml}$ <sup>2</sup>. The low solubility of Diazepam poses a problem while formulating its parental preparation and solubilizing techniques such as: (i) use of cosolvent, (ii) complexation and (iii) altering the pH. Of these the last method is impracticable<sup>3,5</sup>. The complexation agents used for solubilizing Diazepam, such as sodium benzoate and benzoic acid are not universally useful because

of their adverse effects<sup>3,5,6</sup>. Diazepam is known to form complexes with chloral hydrate<sup>7</sup> and bromal hydrate<sup>8</sup> via hydrogen bonding.

With the object of obtaining a stable formulation of Diazepam for parental administration, the present investigation was carried out for the search of a suitable complexing agent for Diazepam without reducing its biological activity. The chemicals were selected for the study on the basis of the reports on their interactions with Diazepam and the theoretical structural considerations. The reagents selected for the study were apparently harmless, could enhance the therapeutic activity of Diazepam and commonly used adjuvants.

"Method of continuous variation" of Job<sup>9</sup> was employed for detection of complexation. The solutions of binary mixtures of Diazepam and the respective agents were prepared by mixing  $1 \times 10^{-5}$  M solutions of the components in distilled water. In the case of Tween 80 and sodium carboxy methyl cellulose, 0.02% w/v solutions were employed. These solutions in 60 ml bottles were equilibrated by shaking for 24 hours in a water bath at  $37^\circ\text{C}$ . At the end of the period, the absorbance at 230 nm, conductance and in some cases the pH, were employed to determine the composition. Examination of the criteria (inflections in absorbance/difference in conductance/pH *versus* molar concentration plots)<sup>10</sup> reveal that the molecular complexes are formed between Diazepam and the chemical agents mentioned in Table I. It can be seen that molar proportions at which the complexes are formed appear different from different methods. Such variations have also been observed by Connors and Mollica<sup>11</sup>.

TABLE I  
Summary of the complexation of Diazepam and other chemical agents

| Sl. No.                  | System containing Diazepam and | Molar ratios indicated by |                     |                     |
|--------------------------|--------------------------------|---------------------------|---------------------|---------------------|
|                          |                                | 6-Spectrophotometry       | Conductometry       | pH                  |
| 1                        | 2                              | 3                         | 4                   | 5                   |
| 1. Sodium Phenobarbitone |                                | 4:1, 3:2, 1:1, 1:4.       | 4:1, 1:1, 3:7.      | 3:7, 1:9, 4:1, 1:1. |
| 2. Chlorpromazine HCl    |                                | 4:1, 3:2, 9:1.            | 4:1, 3:2, 2:3, 1:9. | 4:1, 3:2.           |
| 3. Meprobamate           |                                | 7:3, 3:2, 1:9.            | 3:2, 1:1, 3:7, 1:9. | 4:1, 2:3, 1:9.      |
| 4. Reserpine             |                                | 9:1, 3:2, 2:3, 1:9.       | 9:1, 3:2, 2:3, 1:9. | ..                  |
| 5. Sodium Pantobarbitone |                                | 4:1, 3:7.                 | 4:1, 3:7.           | ..                  |
| 6. Nialamide             |                                | 4:1, 2:3, 1:4.            | 2:3, 1:4.           | ..                  |
| 7. Ascorbic Acid         |                                | 4:1, 2:3, 1:9.            | 4:1, 2:3, 1:9.      | ..                  |
| 8. Chloral Hydrate       |                                | 9:1, 7:3, 1:1, 2:3, 3:7.  | 9:1, 1:1, 3:7.      | ..                  |
| 9. Ephedrine HCl         |                                | 9:1, 7:3, 3:7, 1:9.       | 9:1, 3:7, 7:3.      | ..                  |

TABLE I—(Contd.)

| 1                                    | 2 | 3                       | 4                         | 5  |
|--------------------------------------|---|-------------------------|---------------------------|----|
| 10. Acetaaminophen                   |   | 4: 1, 2: 3, 1: 9, 1: 4. | 9: 1, 1: 3.               | .. |
| 11. Tween 80                         |   | 1: 4.                   | 4: 1, 1: 1, 3: 2, 1: 9.   | .. |
| 12. Sodium Carboxy Methyl Cellulose  |   | 9: 1, 1: 1, 2: 3, 1: 4. | 9: 1, 2: 3.               | .. |
| 13. Serotonin                        |   | 1: 4.                   | 1: 4, 2: 3.               | .. |
| 14. Methyl Parabens                  |   | No Interaction.         | No Interaction.           | .. |
| 15. Propyl Parabens                  |   | 4: 1, 2: 3, 1: 9.       | 4: 1, 3: 2, 2: 3.         | .. |
| 16. Benzalkonium Chloride            |   | 3: 2, 3: 7.             | n4: 1, 1: 1.              | .. |
| 17. Cetyl Trimethyl Ammonium Bromide |   | 4: 1, 1: 1, 1: 4.       | 3: 2, 2: 3.               | .. |
| 18. Cetyl Pyridinium Chloride        |   | 4: 1, 3: 2, 1: 9.       | 4: 1, 3: 2, 1: 9.         | .. |
| 19. Sodium Citrate                   |   | 7: 3, 1: 9.             | 7: 3, 1: 9.               | .. |
| 20. Citric Acid                      |   | 9: 1, 4: 1, 3: 2.       | 3: 2, 2: 3.               | .. |
| 21. Benzoic Acid                     |   | 4: 1, 7: 3, 1: 4.       | 4: 1, 7: 3, 1: 4, 1: 1-7. | .. |
| 22. Sodium Benzoate                  |   | 4: 1, 3: 2, 3: 7, 1: 9. | 4: 1, 3: 2, 3: 7, 1: 9.   | .. |
| 23. Sodium Pyruvate                  |   | 9: 1, 7: 3, 2: 3, 1: 9. | 9: 1, 3: 7, 1: 9.         | .. |
| 24. Lactic Acid                      |   | 4: 1, 1: 1, 2: 3, 1: 4. | 9: 1, 2: 3.               | .. |
| 25. Para Amino Benzoic Acid          |   | No Interaction          | 3: 2.                     | .. |

The complexation reactions of Diazepam and chemical agents mentioned in Table I are being subjected to detailed investigations in this laboratory.

Our thanks are due to University Grants Commission, New Delhi, for the award of a Junior Research Fellowship to one of us (A. B. C.).

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#### EFFECT OF STREPTOLYSIN 'O' ON SUB-CELLULAR ORGANELLE OF TISSUE CULTURE

DURING exposure of tissue cultures to enteroviruses and ultraviolet irradiation, damage to subcellular organelles (lysosomes, mitochondria and plasma membrane, etc.) occurs long before the appearance of the morphological change<sup>1,2</sup>. Cytotoxic changes in tissue cultures are also produced by bacterial toxins<sup>3,4</sup> including streptolysin 'O'<sup>5,6</sup>. In the present study an effort was made to investigate the effect of streptolysin 'O' on the subcellular structures of monkey kidney tissue culture.

Primary monkey kidney tissue culture was prepared in flat bottles. On the 10th day, the fluid was changed with M. Earle's solution without calf serum. Three International Units of streptolysin 'O' (Wellcome

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TABLE I

*Biochemical findings of enzymes in streptolysin 'O' treated MKTC*

| Time    |   | Acid phosphatase (KAU) | Alkaline phosphatase (KAU) | Glutamic oxaloacetic transaminase (Units) | Adenosine triphosphatase (Units) | Lactate dehydrogenase (Units) |
|---------|---|------------------------|----------------------------|---|----------------------------------|-------------------------------|
| 5 min.  | C | 0.53                   | 0.56                       | 27  | 1.06                             | 70                            |
|         | T | 1.04                   | 1.4                        | 14  | 2.13                             | 100                           |
| 10 min. | C | 0.53                   | 0.50                       | 27  | 1.06                             | 70                            |
|         | T | 0.94                   | 1.2                        | 10  | 2.13                             | 120                           |
| 15 min. | C | 0.53                   | 0.45                       | 27  | 1.06                             | 70                            |
|         | T | 1.12                   | 1.5                        | 12  | 2.06                             | 105                           |
| 30 min. | C | 0.53                   | 0.40                       | 26  | 1.02                             | 70                            |
|         | T | 1.32                   | 1.5                        | 22  | 2.93                             | 120                           |
| 1 hr.   | C | 0.53                   | 0.37                       | 25  | 0.80                             | 70                            |
|         | T | 1.55                   | 1.42                       | 27  | 4.26                             | 150                           |
| 2 hrs.  | C | 0.50                   | 0.37                       | 25  | 0.80                             | 65                            |
|         | T | 1.6                    | 1.88                       | 33  | 4.00                             | 165                           |
| 3 hrs.  | C | 0.53                   | 0.37                       | 24  | 0.86                             | 70                            |
|         | T | 0.94                   | 0.95                       | 20  | 3.60                             | 140                           |
| 6 hrs.  | C | 0.50                   | 0.36                       | 26  | 0.93                             | 60                            |
|         | T | 0.82                   | 0.75                       | 24  | 3.20                             | 120                           |
| 24 hrs. | C | 0.50                   | 0.37                       | 27  | 1.07                             | 75                            |
|         | T | 0.94                   | 0.56                       | 20  | 2.40                             | 135                           |
| 42 hrs. | C | 0.53                   | 0.37                       | 27  | 1.07                             | 65                            |
|         | T | 1.05                   | 0.47                       | 22  | 2.13                             | 115                           |
| 72 hrs. | C | 0.75                   | 0.37                       | 26  | 1.06                             | 70                            |
|         | T | 1.12                   | 0.00                       | 20  | 1.67                             | 145                           |

T—streptolysin 'O' treated MKTC.

C—MKTC—not treated with streptolysin 'O'.

Research Laboratory, Backenham, U. K.) was inoculated in each bottle. To the control bottles, equal volumes of the diluent was added in place of streptolysin 'O'. The bottles were incubated at 37° C. The streptolysin 'O' inoculated and the equal number of control bottles were removed at 5, 10, 15 and 30 minutes and 1, 2, 3, 6, 24, 42 and 72 hours and biochemical estimation of acid and alkaline phosphatase and glutamic oxaloacetic transaminase were carried out on the cells as reported earlier<sup>2</sup>. The lactate dehydrogenase was estimated by the colorimetric method of King and Wootton<sup>7</sup>. Adenosine triphosphatase enzyme activity was measured by allowing the 0.5 ml cell homogenate to react on 0.4 ml substrate adenosine triphosphate (0.01 M) in the presence of 0.5 ml tris-buffer (pH 7.4, 0.125 M) and 0.6 ml distilled water for 30 minutes at 37° C. The reaction was then stopped by adding 0.5 ml of 10% trichloroacetic acid. The liberated inorganic phosphorus was determined by the method of Fiske and Subbarow<sup>8</sup>.

The details of the biochemical findings in streptolysin 'O' treated and untreated tissue cultures have been presented in Table I. It was observed that streptolysin 'O' damages the mitochondria (as shown by the increase of adenosine triphosphatase and decrease of GOT), lysosomes (increase of acid phosphatase), plasma membrane (increase of alkaline phosphates) and microsomes (increase of lactate dehydrogenase) of the monkey kidney tissue culture. The increase of the acid phosphatase, adenosine triphosphatase and lactate dehydrogenase started at 5 minutes reaching a peak value between 1 and 2 hours. At later periods elevated levels of these enzymes persisted. Alkaline phosphatase gradually diminished and was not detectable at terminal periods.

The cell damage, as observed in the present study, was the net result of injury to different subcellular organelles and not due to that to any particular structure as suggested by different workers<sup>9-11</sup>. Similar damage to subcellular organelle of monkey kidney tissue culture

has also been produced by viruses<sup>1</sup> and ultraviolet irradiation<sup>2</sup>, in spite of vast difference in their nature. The changes at the molecular or biochemical level start in the cell almost immediately (5 min.) after exposure to all these three types of injury. Though involvement of same subcellular organelles occurred with all the three types of injuries, yet the morphological expression of the injury was totally different. This difference could be due to selective involvement of a particular metabolic pathway in the cell by the injurious agent which may be the deciding factor of the physical expression of the injury.

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## DISINFESTATION OF FRUIT FLIES IN MANGO BY GAMMA IRRADIATION

### Introduction

EARLIER studies in this laboratory have shown that low dose gamma irradiation at 25 Krad either alone<sup>1</sup> or in conjunction with skin coating<sup>2</sup> can delay the ripening process as well as improve the transportability<sup>3</sup> of Alphonso mangoes. The present investigation was undertaken to ascertain the effect of gamma irradiation at dose levels normally employed for delaying the ripening process in mangoes, on the prevention of emergence of adult fruit flies from infested fruits and to establish the radiosensitivity of fruit flies at different stages of their life cycle.

### Materials and Methods

Two fruit fly species, the oriental fruit fly (*Dacus dorsalis*, Hendel) and the melon fly (*Dacus cucurbitae*, Coquilott) commonly found infesting mangoes were selected for these studies. The melon fly was reared under laboratory conditions using pumpkin as the rearing medium. Various life stages of the oriental fruit fly were obtained from a large number of naturally infested mango fruits.

Naked eggs, larvae and pupae of both the species were exposed to 0, 15, 25, 40 and 100 Krad of gamma rays from a cobalt-60 source at a dose rate of 75 Krad/hour. Eggs were carefully transferred to slits made on pumpkin or semi-ripe mangoes as the case may be and irradiated. In some experiments eggs were placed on moist filter paper, and after exposure to the required dose were transferred to fruit slices. Sixty eggs ( $20 \times 3$  replications) each from both the species were exposed to each of the above doses. The experiment was repeated.

Forty larvae ( $20 \times 2$  replications) of 3 to 4 days old from both species were placed on fruit pieces and irradiated with the above doses. The experiment was repeated. After irradiation, the fruit slices containing eggs and larval stages were placed over moist sand in beakers covered with muslin cloth and were held at ambient temperature (25 to 28° C) to determine hatching, larval growth rate, pupation and emergence of adults.

Forty pupae (2 to 3 days old) from each species were exposed to 0 to 100 Krad and were held in moist sand under ambient conditions until adult emergence was complete as judged from the breaking of the puparium at the eclosional suture and partial or full emergence of flies.

### Results and Discussion

In both melon and oriental fruit flies, the percentage of eggs hatched after exposure to 15 and 25 Krad were  $45 \pm 5$  and  $27.5 \pm 2.5$  respectively as against  $75 \pm 5$  in unirradiated control groups (Table I). Irradiation at 40 and 100 Krad prevented hatching of the eggs in both the species. The larvae emerging from eggs exposed to 15 and 25 Krad were rather sluggish and showed slower growth rate as compared to controls. Only  $45 \pm 5\%$  and  $17.5 \pm 2.5\%$  of the emerged larvae formed puparia in 15 and 25 Krad treated groups respectively, as against 100% in controls. No adult emergence was noticed in any of the irradiated groups as against 90% in controls. Irradiating the eggs either on moist filter paper or on fruit pieces did not show any differences in their hatchability.

Larvae exposed to 15 and 25 Krad showed slower growth rates and sluggishness while higher doses caused increased mortality and poorer growth rates. These surviving larvae, after exposure to 40 and 100 Krad,

TABLE I

Effect of gamma irradiation on various life stages of melon and oriental fruit flies<sup>a</sup>

| Developmental stages        | Dose in Krad | <i>Dacus cucurbitae</i> |           |                  | <i>Dacus dorsalis</i> |           |                  |
|-----------------------------|--------------|-------------------------|-----------|------------------|-----------------------|-----------|------------------|
|                             |              | % Eggs hatched          | % Pupated | % Adults emerged | % Eggs hatched        | % Pupated | % Adults emerged |
| Eggs                        | 0            | 75±5                    | 75±5      | 70               | 75±5                  | 75±5      | 70               |
|                             | 15           | 45±5                    | 20±2      | 0                | 27.5±2.5              | 11±1      | 0                |
|                             | 25           | 45±5                    | 9±2       | 0                | 27.5±2.5              | 11±1      | 0                |
|                             | 40           | 0                       | 0         | 0                | 0                     | 0         | 0                |
|                             | 100          | 0                       | 0         | 0                | 0                     | 0         | 0                |
| Larvae<br>(3 to 4 days old) | 0            | ..                      | 87.5±2.5  | 87.5±2.5         | ..                    | 87.5±2.5  | 87.5±2.5         |
|                             | 15           | ..                      | 65±5      | 0                | ..                    | 65±5      | 0                |
|                             | 25           | ..                      | 50        | 0                | ..                    | 50        | 0                |
|                             | 40           | ..                      | 42.5±7.5  | 0                | ..                    | 42.5±7.5  | 0                |
|                             | 100          | ..                      | 0         | 0                | ..                    | 0         | 0                |
| Pupae                       | 0            | ..                      | ..        | 100              | ..                    | ..        | 100              |
| 3 to 4 days old)            | 15 to 100    | ..                      | ..        | 0                | ..                    | ..        | 0                |

<sup>a</sup> Values are expressed as percentage of the initial population taken and are averages of two independent sets of experiments.

did not leave the rearing medium for pupation. In the case of larvae from both the species subjected to 0, 15, 25, 40 and 100 Krad, the percentage pupation was 87.5±2.5, 65±5, 50, 42.5±7.5 and 0 respectively (Table I). While 100% adults emerged from unirradiated groups no adult emergence occurred in any of the irradiated groups.

In the case of pupae, irradiation at doses as low as 15 Krad completely prevented adult emergence in both the species. Burditt and Seo<sup>4</sup> have reported that the emergence of adults from irradiated naked eggs or larvae or from puparia containing fourth instar larvae of the mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and the melon fly was prevented by 15 Krad of gamma irradiation, but larger doses were required to prevent emergence of irradiated pupae.

Gamma irradiation disinfection of mangoes has the added benefit of delaying of the ripening process as well over the more conventional methods of disinfection, such as ethylene dibromide fumigation or vapour heat sterilization. Moreover these methods have rather narrow margins between the dosages that are required to destroy insect infestations and the dosages that will cause injury in the treated produce<sup>5</sup>. Another advantage of irradiation could be that mangoes infested by the mango seed weevil [*Sternuchus mangiferae* (F)], which does not succumb to fumigation or other known quarantine treatments, can be sterilized with about 20.6 Krad of gamma rays<sup>6</sup>, which is about the same dosage effective against fruit flies as well as for delaying the ripening processes.

The results of these preliminary studies show that a dose of 25 Krad of gamma rays recommended for delaying the ripening of mangoes, can prevent the emergence of adults from fruit infested with the different developmental stages of oriental and melon fruit flies.

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**CHROMOSOMES OF THE MOLE CRICKET**  
***GRYLLOTALPA FOSSOR* SCUDDER**  
(ORTHOPTERA)

THE only information available on the chromosomes of the species *Gryllotalpa fossor* is of Asana *et al.* (1940) and Kushnir (1952). We present in this report some interesting observations on the behaviour of the chromosomes during meiosis with special reference to the X chromosome. Chromosomes from testis or ovary were prepared from nearly 150 animals by the usual squash or air dry technique.

The diploid chromosome number is 23 in male and 24 in the female (Fig. 1). The autosomes comprise 9 pairs of metacentric chromosomes and 2 pairs

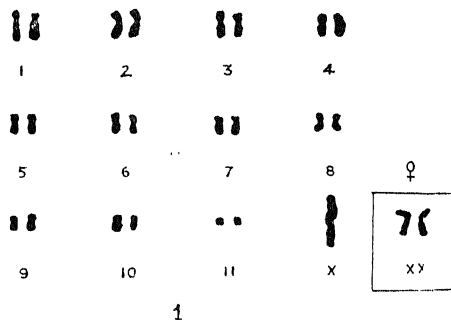
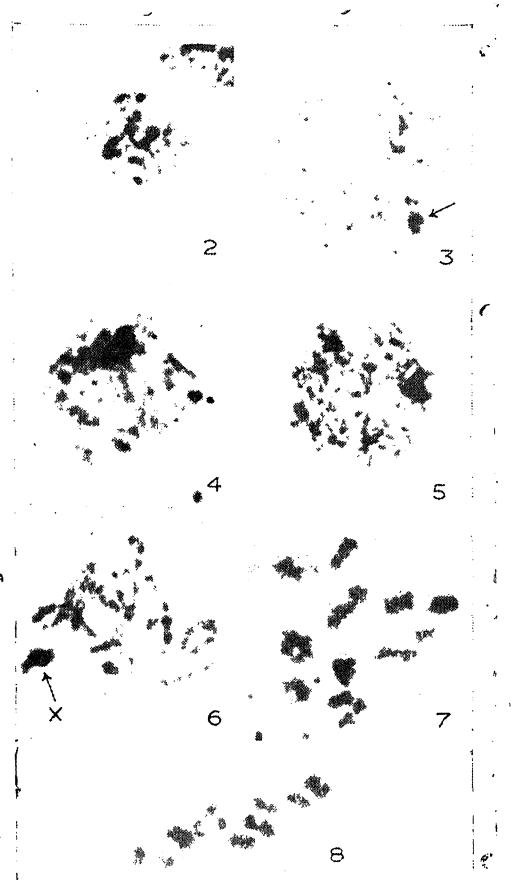


Fig. 1. Karyotype from gonial metaphase plate, showing 9 pairs of metacentric and 2 pairs of sub-metacentric chromosomes. X chromosome is metacentric and largest of the complement. Inset showing sex (XX) chromosomes from female oögonial plate.

of sub-metacentric chromosomes. There is a single X chromosome (XO) in the male and two X (XX) chromosomes in the female. The X chromosome is the largest metacentric chromosome with arm ratio of 1.01 and constitutes about 18% of total haploid complement length. Supernumerary chromosomes are not observed in any of the specimens studied. The interphase nuclei of somatic cells of male and female show (1 to 3) chromocenters (Fig. 2). In the spermatogonial interphase one prominent chromocenter, probably sex chromatin body, is conspicuous (Fig. 3). At Leptotene, zygotene and early pachytene, there are two small chromocenters in addition to the prominent chromocenter (X chromatin body?) (Figs. 4, 5). The homologous and non-homologous chromosomes are associated in the formation of these chromocenters. One pair of autosome is deeply stained, and its heteropycnotic nature is clearly recognisable at diplotene and diakinesis. At late pachytene the X chromosome shows two regions, a highly

condensed part and a distal isopycnotic segment (Fig. 6).



Figs. 2-8. Fig. 2. Interphase nucleus from malpighian tubules of female mole cricket. Note the number of chromocenters,  $\times 1,400$ . Fig. 3. Spermatogonial interphase nucleus. Note the prominent (arrow) peripheral heterochromatic body,  $\times 1,400$ . Fig. 4. Leptotene,  $\times 1,400$ . Fig. 5. Zygotene. Note the prominent and other chromocenters,  $\times 1,400$ . Fig. 6. Pachytene. Note the differential staining of X chromosome,  $\times 1,400$ . Fig. 7. Diakinesis,  $\times 1,400$ . Fig. 8. Metaphase,  $\times 1,400$ .

All the autosomes show fuzzy nature during early meiotic prophase. No projections are observed in the darkly stained bivalent at diakinesis (Fig. 7). At diplotene and diakinesis the projections are few and prominent (Fig. 7). At metaphase I (Fig. 8) all the chromosomes are highly condensed. The isopycnotic area of the X chromosome also exhibits a fuzzy nature at leptotene, zygotene and pachytene, whereas at diplotene and diakinesis as the X



TABLE I

Chromosome number and sex determining mechanism of *Gryllotalpidae*

| Species   | (2n) Male                                   | (2n) Female                                       | Sex chromosome<br>mechanism<br>Male/Female | Reference*  |
|---|---|---|--|---|
| <i>Gryllotalpa fossor</i>                                   | 23 : 22a+X<br>24 : 22a+s+X<br>25 : 22 +2s+X | 24 : 22a+XX<br>25 : 22a+s+ XX<br>26 : 24a+ 2s+ XX | XO/XX                                      | Asana <i>et al.</i> , 1940;<br>Ohmachi, 1929,<br>1935 |
| <i>Gryllotalpa borealis</i>                                 | 23 : 22a+X                                  | 24 : 22a+XX                                       | XO/XX                                      | Payne, 1912, 1916                                     |
| <i>Gryllotalpa gryllotalpa</i><br>Belgium, France, N. Italy | 12 : 10a+XY                                 | ..  | XY/—                                       | Payne, 1916;<br>Barigozzi, 1923                       |
| S. Italy  | 15 : 14a+XO                                 | ..  | XO/—                                       | ..  |
| Milano  | 18  | ..  | ..   | Barigozzi, 1947                                       |
| Romania   | 14 : 12a+XY<br>15 : 12a+s+XY                | ..  | XY/—<br>XY/—                               | Steopoe, 1939<br>..                                   |
| <i>Gryllotalpa vulgaris</i><br>Freiburg                     | 12 : 10a+XY                                 | ..  | XY/—                                       | Payne, 1916;<br>Voinov, 1925                          |
| Bucharest   | 14 : 17                                     | ..  | ..   | ..  |
| Italy   | 17 : 16a+X                                  | ..  | XO/—                                       | ..  |
| Romania   | 14 : 12a+XY                                 | ..  | XY/—                                       | ..  |

\* Data collected from Asana *et al.*, 1940; Kushnir, 1952.

chromosomes start condensing the isopycnotic part is not recognisable.

The chromosome number reported here is in agreement with those of Asana *et al.* (1940) and Kushnir (1952). However, our description of the karyotype (9 pairs of metacentric chromosomes, 2 pairs of sub-metacentric chromosomes and metacentric X chromosome) differs from that given by Asana *et al.* (1940) where the karyotype consists of all V-shaped (metacentric?) chromosomes. Contrary to earlier findings supernumerary chromosomes are not present in the population. These chromosomes might have been lost during the karyotype evolution or have become incorporated with the autosomes.

The Gryllotalpidae is a small family comprising a single genus with 50 species of which chromosome studies have been done on only 5 species. *G. fossor* and *G. borealis* have 23 chromosomes in male and 24 in female (Table I). In *G. gryllotalpa* and *G. vulgaris* the diploid number varies from 12 to 18. From the variations in chromosome number it appears that the origin of these two chromosome types is divergent. White (1954) is of the view that the primitive ancestors of the family had 23 chromosomes and the lower number is derived by reduction of the chromosome number. Kushnir (1952) holds the opinion that the original group had lesser number and the higher number might have reached by doubling and readjustment of the sex determining mechanism.

With the demonstrations of repeated DNA sequence of centromeric heterochromatin in most organisms, it is most likely that the simple mechanism suggested by White (1954) centric fusion is operative.

In *G. fossor* the X chromosome shows linear differentiation at meiotic prophase stage. The distal part of one arm is isopycnotic, and the rest of the chromosome shows positive heteropycnosis. In the majority of Orthoptera, so far known, the entire X chromosome shows positive heteropycnosis. This partial allocycly of the X chromosome in this animal, showing a distal isopycnotic segment in one arm, indicates that this part may be genetically active.

We thank Mr. E. A. Daniels for microphotography.

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# THE CHROMOSOME NUMBER OF *CYATHOCLINE LYRATA* CASS.

*Cyathocline* is represented by three species in India (Santapau and Henry<sup>1</sup>). Of these, *Cyathocline lyrata* alone has drawn the attention of cytologists. Koul<sup>2</sup> and Mehra *et al.*<sup>3</sup> put on record the chromosome number of *Cyathocline lyrata* Cass. as  $2n = 22$ . Incidentally, this is the only count known so far, for the entire genus (Fedorôv<sup>4</sup>).

While surveying chromosome numbers of the angiosperms of Jammu and Kashmir State, the authors worked out the chromosome number of three populations of *Cyathocline lyrata*. The counts were made from acetorcein squashes of fresh and fixed flower buds at various stages of pollen mother cell meiosis.

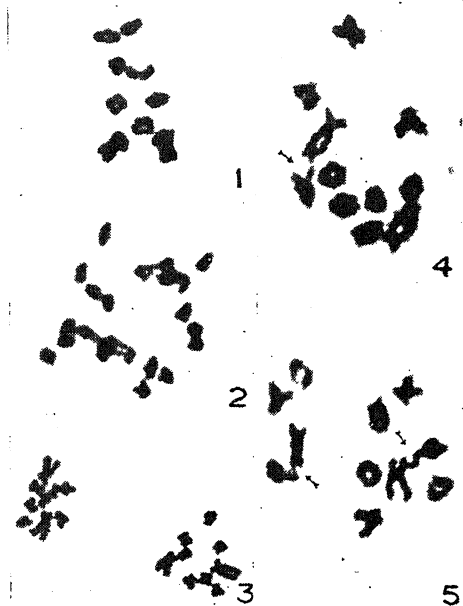
Counting is easily possible at metaphase and anaphase-I (Figs. 1 and 2) when the chromosomes are deeply stained and greatly condensed and therefore, spread out with ease. All cells scored at these two stages had  $2n = 18$ . The nine metaphase bivalents are of two sizes; 2 long and seven small (Fig. 1). While the smaller bivalents have only one or two chiasmata the two big bivalents very often have three chiasmata each; two in the long arm and one in the short arm. On account of longer size and a higher number of chiasmata, chromosomes of the big bivalents almost invariably separate out much later than those of the smaller bivalents (Fig. 2). The size difference is maintained even after the chromosomes have reached the poles.

From anaphase-I chromosomes directly enter into metaphase-II. As a result, they continue to keep condensed and show off their centromeres very distinctly (Fig. 3). The two long chromosomes have subterminal constrictions. Of the seven small chromosomes two have median and five submedian constrictions. The karyotype is asymmetric.

Pollen viability determined by the inorganic acid test (Koul and Paliwal<sup>5</sup>) is fairly good, so also is the seed set indicating that the plants are sexually fertile.

Four populations of *Cyathocline lyrata* are known with  $n = 11$  (Koul<sup>2</sup>; Mehra *et al.*<sup>3</sup>). The present count determined from three Jammu populations represents a second count for the species, making it dibasic. Before accepting this as final, one should, however, not lose sight of the fact that the earlier count of  $n = 11$  has been determined at diakinesis, which in the present species does not seem to represent an ideal stage for making chromosome counts. From what has been observed in the present material, the prophase chromosomes show differentiation into eu- and heterochromatin. This is particularly true of the large sized bivalents, in which the deeply stained terminal ends are interspersed by the long faintly stained regions. At times, when the intercalary region is not distinct, the terminal ends of the bivalents look like two separate

"bivalents" (Figs. 4 and 5). Thus, the two long bivalents can be mistaken for four.



FIGS. 1-5. Fig. 1. A microspore mother cell at metaphase-I bearing 9 II,  $\times 1,500$ . Fig. 2. A microspore mother cell at anaphase-I showing two late separating bivalents,  $\times 1,500$ . Fig. 3. A microspore mother cell at late anaphase-I showing 9 chromosomes at each pole,  $\times 1,500$ . Fig. 4. A microspore mother cell at diakinesis showing 9 II. One of the longer bivalents showing a breakage,  $\times 1,500$ . Fig. 5. A microspore mother cell at diakinesis showing 9 II of which the long bivalents show breakage  $\times 1,500$ . (Arrows in Figs. 4 and 5 indicate the possible sites of breakage in the long bivalents.)

Similar effect is also brought about by the breakage of the two long bivalents under the pressure of squashing. In this way ten or eleven "bivalents" could be formed. This suggests that  $n = 11$ , perhaps represents only an artefact. This is also borne out by the fact that in cells with more than nine "bivalents", the size difference between the bivalents gets lost. All the eleven "bivalents" are uniform in size, suggesting that the species has a symmetric karyotype with respect to chromosome size (which it has not). While this is one possibility the other can be that *Cyathocline lyrata* really exists in two races, one with  $n = 9$  (reported here) and the other with  $n = 11$  (reported by Koul<sup>2</sup> and Mehra *et al.*<sup>3</sup>).

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#### A NEW ROOT APHID ON SUGARCANE IN SOUTH INDIA

THE occurrence of aphids in the roots of sugarcane is a rare phenomenon in India. George (1925, 1928) recorded *Tetraneura cyanodonti* var. *Coimbatorensis* George, now known as *T. javensis* v.d. Goot from sugarcane in Coimbatore. Later Nagarajan (1957) described this aphid damage to sugarcane around Coimbatore. Fletcher (1928) reported the occurrence of another species, *Geocia spatulata* Theob. from sugarcane at Pusa.

Recently in the course of field investigations on the white grub damage to sugarcane at the Sugarcane Breeding Institute Farm, another species of aphid, *Forda* (*Pentaphis*) *orientalis* George has been observed to damage sugarcane roots. The affected plants, generally ratoons of 8 months age, showed stunted growth and typical symptoms of mosaic on their leaves. The varieties affected are Co. 419 and Co. A. 71-1. Colonies of creamy-white, hemispherical aphids were present in these roots. This species of aphid (Fig. 1)

may be distinguished from *T. javensis* by the absence of long hairs and cornicles. These aphids were present at a depth of 7 to 20 cm below soil surface. Ants were found to be attendant on them. Border rows of cane were heavily infested. The aphids were found to infest the canes during July-September.



FIG. 1 (b). The root-aphid, *Forda orientalis* George.

Earlier, this species has been reported to occur only on sorghum and bajra in Coimbatore by George (1928) and David (1958 and 1969) and on *Bathriochloa insculpta* at Dehra Dun by David (1969). This is the first record of the occurrence of this aphid as a pest in the sugarcane crop.

Our thanks are due to Dr. S. K. David, Retired Entomologist, Agricultural College, Coimbatore, for kindly identifying the aphid and also to the Director and Entomologist, Sugarcane Breeding Institute, Coimbatore, for giving facilities and also help in the preparation of this note.

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FIG. 1 (a). Sugarcane roots with root-aphids attached.

# GENUS *CHAETOPELTIS* BERTHOLD<sup>1</sup> IN INDIA

EVER SINCE Berthold's<sup>1</sup> creation of *Chaetopeltis* as a new genus, there have been very few reports on the occurrence of the alga. The genus comprises of two well-defined species, viz., *Chaetopeltis orbicularis* Berthold and *Chaetopeltis barbata* (Bohlin) Wille. A third species, *Chaetopeltis americana* (Snow) Collins<sup>3</sup>, has been considered by some authors as belonging to a different genus, *Pseudulvella* (Smith, 1933<sup>2</sup>). Since the genus is, hitherto, unknown from India, it is intended here to record it in our flora and briefly describe the salient features of the Indian plant.

Twigs with epiphytic *Stigeoclonium* were collected in December 1974 from a small puddle near Chinhat village about six miles from Lucknow on Fyzabad Road, washed with boiled, cooled tap water and placed in Chu's solution no. 10<sup>2</sup>, fortified with 5% soil extract. Two or three days after inoculation, a mixed growth of *Stigeoclonium* sp. and *Chaetopeltis orbicularis* was observed forming bright green patches on the bottom of the vessels. In subsequent sub-cultures, the alga was separated from the *Stigeoclonium* species and other contaminants and established in unialgal cultures.

The thallus of the Indian plant is made up of a small monostromatic disc bearing very fine, delicate, mucilaginous bristles on its upper surface (Figs. 1-5). The

cells are uninucleate, 8-15  $\mu$ m in diameter and possess a single, irregularly thickened, parietal laminate chloroplast with a single pyrenoid. In older thalli, most of the cells lose their contents owing to the formation of zoospores (Figs. 6). The present form agrees closely with *Chaetopeltis orbicularis* f. *minor* Möbius<sup>4</sup> in thallus organization, cell-structure, dimensions and behaviour in cultures and is, therefore, identified as the same.

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## EFFECT OF X-RAYS ON DEVELOPING EMBRYOS OF RICE (*O. SATIVA* L.) AND THEIR MUTATION SPECTRUM

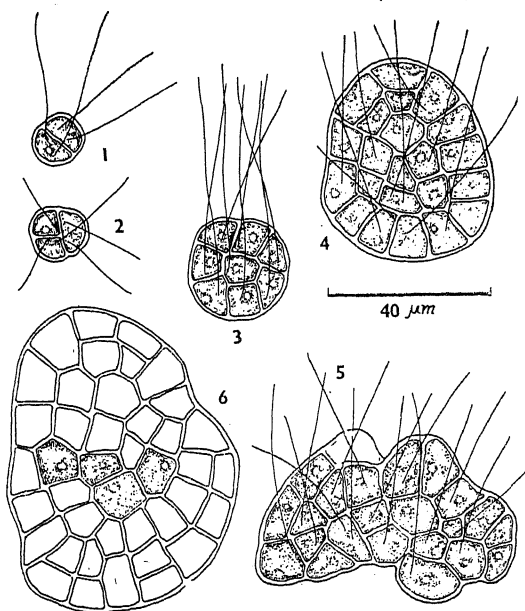
EFFECT of X-rays on rice seeds and excised mature and immature embryos have been studied extensively in this laboratory<sup>1-3</sup>. To understand the effect of X-rays on developing embryos, proembryos of a high yielding rice variety (IR-8) were subjected to different doses of X-rays.

X-ray doses of 2.5, 5.0 and 7.5 Kr were applied to the 100 flowers at 5 hours (one celled embryo, Endosperm nucleus starts dividing), 8 hours (Two celled embryo), 12 hours (Four celled embryo), and 24 hours (Generally eight celled embryo) after pollination. Following irradiation, the proembryos were allowed to grow on maternal tissue. After maturation the embryos were first grown *in vitro* in White's agar medium up to 28 days and then transplanted in the field. At maturity panicles from the main tillers of all X<sub>1</sub> plants were harvested separately. X<sub>2</sub> generation was raised as panicle to progeny row.

Effect of radiation was noted from the data of seed setting percentage. Considerable degree of sterility was noticed in all the treatments, as compared to control (Table I). Sterility percentage increased with the increase of doses. It was maximum at 5 hours after pollination and gradually declined in next stages of development (Table I).

Reduction in germination percentage was noticed among the irradiated embryos, excepting in 5 hours after pollination where cent per cent germination was found at 2.5 Kr like control (Table I).

Seedling lethality was noticed from the 5th day onwards in tube culture as compared to control.



FIGS. 1-6. *Chaetopeltis orbicularis* f. *minor* Möbius. Figs. 1-3. Early vegetative stages of growth of thallus. Figs. 4-5. Mature vegetative thallus with bristles. Fig. 6. Old thallus showing empty cells from which the swimmers have escaped out and the absence of mucilaginous bristles.

TABLE I  
*Radiosensitivity of rice following X-ray irradiation to proembryos*

| Plant attributes     | Control | 5 hours* |         |         | 8 hours |         |         | 12 hours |         |         | 24 hours |         |         |
|----------------------|---------|----------|---------|---------|---------|---------|---------|----------|---------|---------|----------|---------|---------|
|                      |         | 2.5 Kr.  | 5.0 Kr. | 7.5 Kr. | 2.5 Kr. | 5.0 Kr. | 7.5 Kr. | 2.5 Kr.  | 5.0 Kr. | 7.5 Kr. | 2.5 Kr.  | 5.0 Kr. | 7.5 Kr. |
| % Sterility          | 7.2     | 51.4     | 74.5    | 81.5    | 30.4    | 52.7    | 70.8    | 26.9     | 45.1    | 50.9    | 25.8     | 41.6    | 49.8    |
| % Germination        | 100.0   | 100.0    | 97.3    | 93.4    | 100.0   | 96.8    | 92.5    | 100.0    | 93.9    | 90.1    | 100.0    | 87.8    | 83.2    |
| % Seedling Lethality | ..      | 60.4     | 72.4    | 100.0   | 54.6    | 66.0    | 94.7    | 48.1     | 53.8    | 70.5    | 26.0     | 46.5    | 61.2    |

\* Hours after pollination.

TABLE II  
*Mutation frequencies\* (in percentage) of rice following X-ray irradiation to proembryos*

| Different stages of proembryo growth (Hrs. after pollination) | 2.5 Kr.     |               | 5.0 Kr.     |               | 7.5 Kr.     |               |
|---|-------------|---------------|-------------|---------------|-------------|---------------|
|   | Chlorophyll | Morphological | Chlorophyll | Morphological | Chlorophyll | Morphological |
| 5 hours   | 0.21        | 20.0          | 1.73        | 18.7          | ..          | ..            |
| 8 hours   | 0.24        | 17.5          | 1.67        | 15.3          | 1.72        | 10.5          |
| 12 hours  | 0.27        | 16.8          | 1.70        | 15.4          | 1.65        | 9.7           |
| 24 hours  | 0.35        | 16.5          | 2.13        | 12.7          | 1.85        | 7.5           |

\* Mutation frequency was calculated on the basis of No. of mutants per 100  $X_2$  plants.

Lethality increased with increase of dosage. Cent per cent seedling lethality was noticed in 5 hours after pollination at 7.5 Kr (Table I).

Mericle and Mericle<sup>5-7</sup> obtained highest mutation frequency in  $R_2$  generation following proembryo irradiation. Kawai and Inoshita<sup>4</sup> obtained higher mutation spectrum by irradiating during meiosis and heading time. In this experiment besides chlorophyll mutations, other morphological mutations were also scored from a population of 2000  $X_2$  plants raised from the seeds of 120  $X_1$  panicles. The frequency of chlorophyll mutations was found to be maximum in 24 hours after pollination at 5.0 Kr (Table II). In the four stages of proembryo growth the frequency of morphological mutations were found to be maximum at the lower doses which decreased with the increase of doses (Table II). This experiment shows that comparatively lower doses of X-rays can be effectively used for induction of mutation if treated at the proembryonic stages.

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**PROMOTIVE EFFECT OF NaCl AND CaCl<sub>2</sub>  
MIXTURES AND CaCl<sub>2</sub> AND MgCl<sub>2</sub> MIXTURES  
ON EXTENSION GROWTH OF GUAR  
SEEDLINGS**

Most of the studies on seed germination and salinity have been on emergency of seedlings, seedling growth and their tolerance to different concentrations of salts—NaCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, NaHCO<sub>3</sub>, etc.<sup>1-4</sup> A few studies relate the combined effects of NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> as mixtures on germination etc.<sup>5-7</sup> The present work was undertaken to investigate the effects of NaCl, CaCl<sub>2</sub> and MgCl<sub>2</sub> singly as well as the mixtures of NaCl and CaCl<sub>2</sub> and CaCl<sub>2</sub> and MgCl<sub>2</sub> on the root and the shoot growth of guar seeds.

Ten uniform and graded seeds of guar *Cyamopsis psoralides*, L. Cv. Nau Bahar were germinated in petridishes on Whatman filter-paper No. 1. The germination media (5.00 ml) were distilled water (DW) and saline media—NaCl, CaCl<sub>2</sub> and MgCl<sub>2</sub>, (0.25, and 0.50%). Besides, a mixtures of NaCl and CaCl<sub>2</sub>, and MgCl<sub>2</sub> and CaCl<sub>2</sub> of the above solutions in 1:1, 3:1 and 1:3 ratios were also used. The actual concentrations of the above mixtures were in 0.25% solutions 0.125% NaCl or MgCl<sub>2</sub> + 0.125% CaCl<sub>2</sub>—1:1, 0.1875% NaCl or MgCl<sub>2</sub> and 0.0625% CaCl<sub>2</sub>—3:1 and 0.0625% NaCl or MgCl<sub>2</sub> and 0.1875% CaCl<sub>2</sub>—

1:3. Concentrations were double in 0.50% series, and the total volume was kept constant at 5.00 ml. Germination experiment was carried out in day light at room temperature  $28 \pm 2^\circ \text{C}$ . In all, 6 such sets were run and root length and shoot length were measured from seedlings at 96 hours of germination. Mean data are reported and are in comparison with those indistilled water medium.

**Root length (Table I).**—Extension growth of roots was suppressed by NaCl and MgCl<sub>2</sub>. Maximum and minimum reductions were caused respectively by NaCl and CaCl<sub>2</sub>. 0.5% CaCl<sub>2</sub> increased root length. Root length was increased by the mixture of NaCl and CaCl<sub>2</sub> and CaCl<sub>2</sub> + MgCl<sub>2</sub>, being maximum in 0.5% NaCl and CaCl<sub>2</sub> 3:1 mixture—in fact, the retardation by NaCl and MgCl<sub>2</sub> was reversed by this mixture. Mixtures of CaCl<sub>2</sub> and MgCl<sub>2</sub> 0.25 and 0.5% 1:1, 1:3 also promoted root length.

**Shoot length (Table II).**—Shoot length was suppressed by all salts, the suppression being greater by NaCl. Mixtures of CaCl<sub>2</sub> and MgCl<sub>2</sub> 0.25 and 0.5%—1:1 and 1:3 as well as CaCl<sub>2</sub> + NaCl 0.25% 1:1 reversed the suppression by individual salts, and shoot length was almost equal to that in distilled water. Thus, each salt alone exerted a suppressing effect on the root and shoot length. However, mixtures with CaCl<sub>2</sub>

TABLE I  
Root Length—Cm (at 96 hours)

| Sl. No. | Treat-ment     | Distilled water | NaCl           | CaCl <sub>2</sub> | MgCl <sub>2</sub> | NaCl + CaCl <sub>2</sub> 1:1 | NaCl + CaCl <sub>2</sub> 1:3 | NaCl + CaCl <sub>2</sub> 3:1 | CaCl <sub>2</sub> + MgCl <sub>2</sub> 1:1 | CaCl <sub>2</sub> + MgCl <sub>2</sub> 1:3 | CaCl <sub>2</sub> + MgCl <sub>2</sub> 3:1 |
|---------|----------------|-----------------|----------------|-------------------|-------------------|------------------------------|------------------------------|------------------------------|---|---|---|
| 1.      | Control (D.W.) | 8.75<br>±0.26   | ..             | ..                | ..                | ..                           | ..                           | ..                           | ..  | ..  | ..  |
| 2.      | 0.25%          | ..              | 5.85<br>*±0.36 | 10.35<br>±0.45    | 6.10<br>±0.44     | 10.90<br>±0.52               | 9.90<br>±0.41                | 10.10<br>±0.40               | 12.00<br>±0.51                            | 8.70<br>±0.45                             | 10.50<br>±0.27                            |
| 3.      | 0.50%          | ..              | 2.65<br>±0.21  | 10.35<br>±0.48    | 2.30<br>±0.29     | 9.70<br>±0.41                | 8.75<br>±0.20                | 12.15<br>±0.53               | 11.20<br>±0.39                            | 11.65<br>±0.51                            | 8.20<br>±0.30                             |

\* S.E. of Means.

TABLE II  
Shoot Length—Cm (at 96 hours)

| Sl. No. | Treat-ment | Distilled water | NaCl          | CaCl <sub>2</sub> | MgCl <sub>2</sub> | NaCl + CaCl <sub>2</sub> 1:1 | NaCl + CaCl <sub>2</sub> 1:3 | NaCl + CaCl <sub>2</sub> 3:1 | CaCl <sub>2</sub> + MgCl <sub>2</sub> 1:1 | CaCl <sub>2</sub> + MgCl <sub>2</sub> 1:3 | CaCl <sub>2</sub> + MgCl <sub>2</sub> 3:1 |
|---------|------------|-----------------|---------------|-------------------|-------------------|------------------------------|------------------------------|------------------------------|---|---|---|
| 1.      | Control    | 6.60<br>±0.16   | ..            | ..                | ..                | ..                           | ..                           | ..                           | ..  | ..  | ..  |
| 2.      | 0.25%      | ..              | 4.80<br>±0.13 | 6.55<br>±0.18     | 6.05<br>±0.31     | 7.25<br>±0.15                | 6.95<br>±0.20                | 5.85<br>±0.21                | 6.20<br>±0.27                             | 6.15<br>±0.22                             | 6.00<br>±0.18                             |
| 3.      | 0.50%      | ..              | 3.20<br>±0.13 | 5.50<br>±0.19     | 3.25<br>±0.22     | 5.75<br>±0.22                | 4.95<br>±0.25                | 5.40<br>±0.15                | 6.00<br>±0.14                             | 6.20<br>±0.10                             | 5.40<br>±0.16                             |

enhanced the length and reversed the suppression. As early as 1903, Loew<sup>8</sup> suggested that Ca neutralizes the toxic effects of single salts of Na, K and Mg and a certain ratio of Ca and Mg was necessary for the proper growth of plants. It is also known that certain combinations and ratios of salts or ions are more beneficial to plant growth than others.<sup>9-11</sup> This is borne out from the data reported here. Beneficial effect of mixture of NaCl and CaCl<sub>2</sub> may be attributed to the fact that NaCl increases succulence and hydration, while Ca is a constituent of middle lamella of cells and promotes root growth<sup>12</sup>. When combined, there is increased hydration and availability of cell wall material; this results in the increased root length. Retardation of shoot length caused by individual salts is also reversed by the mixtures, perhaps the ratios 1:1 and 1:3 may balance the toxic effects of individual ions.<sup>8-10</sup> Mg is required for rapid growth of young cells, high protein concentrations and active mitosis, it is also an enzyme activator for carbohydrate metabolism and is associated with the energy supplying phosphorus compounds.<sup>12</sup> In combination with Ca any of the effects of Mg enumerated above may be stimulated or augmented and thereby, alleviate retardation by their individual toxic effect.

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# A NOTE ON TOXICITY OF *NICOTIANA GOSSEI* DOM. TO THE LARVAE OF TOBACCO STEM BORER *GNORIMOSCHEMA HELIOPA* LOW.

MANY *Nicotiana* species have been reported to be resistant to various insect species. Thurston (1961)<sup>3</sup> reported that *Nicotiana gossei* Domin; *N. repanda* Willd.; and *N. trigonophylla* Dun. were highly resistant to green peach aphids. Parr and Thurston (1968)<sup>4</sup> have reported the toxicity of *Nicotiana gossei* to the larvae of tobacco hornworm *Manduca sexta* Joh. Burk and Stewart (1969)<sup>2</sup> also have reported the resistance of *Nicotiana gossei* to green peach aphids. The toxicity of *N. gossei* to green peach aphids (*Myzus persicae* Sulz.) and first three instar larvae of tobacco leaf eating caterpillar (*Spodoptera litura* F.) in the laboratory was reported from C.T.R.I. (Anonymous, 1972-73).<sup>1</sup> In India, the tobacco stem borer *Gnorimoschema heliopa* Low is a serious pest on Virginia, bidi and other types of tobacco. Hence in the present investigation *Nicotiana gossei* which has been reported toxic to various insects has been screened for resistance to *G. heliopa* Low in the laboratory.

The culture of *G. heliopa* was maintained in the laboratory. *Nicotiana gossei* and *N. glauca* Grah. used in the test were grown in the glass house; the latter species was used as non resistant check. Five freshly laid eggs of *G. heliopa* were transferred to the tender leaf. Five plants of both the species of *Nicotiana* were used for testing. After the eggs hatched, daily observations on feeding of first instar larvae and their survival were recorded. The trial was repeated after about 15 days of completion of the first trial.

Observations indicated that the eggs hatched within 3 days. Immediately after hatching, the larvae start crawling on the trichomes and nibbling the leaf tissue. The larvae feed on the epidermis of the leaf tissue of *N. gossei* (Fig. 1). All the twenty-five larvae that hatched on *N. gossei* showed nervous convulsions and morbidness within 24 hours. They dehydrated very

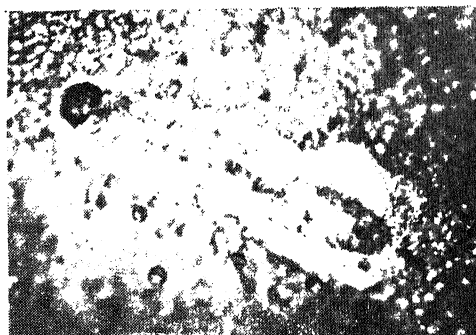


FIG. 1. Epidermal feeding of first instar larva of *G. heliopa* L. on *N. gossei* D.  $\times 50$ .

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rapidly and became brittle after death. Against this, the larvae fed profusely on *N. glauca* and developed fully. Similar results were obtained when it was repeated for a second time in the laboratory with the same number of *G. heliopa* eggs per plant. These observations clearly indicate that *N. gossei* is highly toxic to first instar larvae of *G. heliopa*; thus serving as a good source of resistance to tobacco stem borer.

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#### INTERACTION BETWEEN CYCOCEL AND GIBBERELIC ACID IN POLLEN TUBE ELONGATION OF *CALOTROPIS PROCERA*

RECENTLY, the effect of various growth regulators, e.g., abscisic acid (ABA), cycocel (CCC), gibberellic acid ( $GA_3$ ), indole acetic acid (IAA) and kinetin (K) on pollen tube elongation was studied by us<sup>1</sup>. It was observed that IAA and CCC inhibited the elongation of pollen tubes while the rest of the growth substances regulated additional elongation. Interaction between these growth substances in respect of tube elongation was also studied. When used in combination, some of these growth regulators exhibited synergism, (e.g., CCC +  $GA_3$ ). Present studies describe this interaction.

Pollinia were dissected out from the flowers of *Calotropis procera* and incubated in 15% sucrose medium in cavity slides. Different concentrations of cycocel (CCC) and gibberellic acid ( $GA_3$ ) were maintained in 15% sucrose as a basal medium under constant illumination from a light bank of two 40W fluorescent lamps at a distance of  $\frac{1}{2}$  meter. The controls were maintained in a basal medium excluding these compounds. Incubation was carried out at  $28^\circ C \pm 2^\circ C$  for a period of 4 hours. In each culture, 3 pairs of pollinia were incubated and 3 replicates were run in each treatment. Mean length of 20 pollen tubes was recorded for each treatment,

and on the basis of results obtained, standard deviation was calculated.

When CCC was applied to the pollinium, it retarded the elongation of pollen tubes. However,  $GA_3$  at 10 ppm enhanced the growth of the tubes to the maximum. Higher concentrations of  $GA_3$  did not produce a linear response (Table I). An

TABLE I  
Interaction between CCC and  $GA_3$  on the elongation of Pollen Tubes

The concentrations are given as ppm

(a) 4 h of treatment.

(b) 1 h of pretreatment, 3 h post-treatment.

| Treatment              |        | Length of P.T. ( $\mu$ ) |
|------------------------|--------|--------------------------|
| CCC                    | $GA_3$ | ..                       |
| 10a                    | ..     | 380 $\pm$ 10.9           |
| 25a                    | ..     | 325 $\pm$ 10.6           |
| ..                     | 10a    | 570 $\pm$ 19.2           |
| ..                     | 20a    | 490 $\pm$ 15.2           |
| 10                     | 10b    | 366 $\pm$ 10.9           |
| 10                     | 20b    | 480 $\pm$ 7.4            |
| 10                     | 30b    | 425 $\pm$ 7.95           |
| 25+                    | 10a    | 1100 $\pm$ 46.1          |
| Control (15% sucrose)a |        | 452 $\pm$ 15.5           |

attempt was made to examine the interactive effects of  $GA_3$  and CCC in relation to pollen tube elongation. Interestingly enough, combined application of the two had evidently additive or synergistic effect, resulting in more growth than the controls, or when either compound especially  $GA_3$  was used. The maximum elongation was obtained with the optimum proportions of  $GA_3$ /CCC in the mixture as 10/25 ppm (1100  $\mu$ ) or 25/5 ppm (960  $\mu$ ). Even proportions such as 5/5 (690  $\mu$ ), 10/10 (760  $\mu$ ), 25/10 (730  $\mu$ ) produced greater elongation of pollen tubes. The elongating effect of  $GA_3$  is known in many plant organs (Audus, 1972)<sup>1</sup>. It is widely known that  $GA_3$  reverses the retarded growth caused by many retardants<sup>2,3</sup>, except in barley endosperm test, where Briggs (in Audus, 1972) failed to antagonise the effect of  $GA_3$  by cycocel. It is widely known that CCC acts at the level of gibberellins biosynthesis<sup>3</sup>. The fact that cycocel, in the manner of an 'antigibberelline', suppressed the elongation of tubes, strongly suggested that native gibberelline(s) existed and/or were biosynthesised during the germination of pollen tubes under natural conditions. Our present studies also show that the post-treatment with exogenous  $GA_3$  restored the retarding effect of CCC. In addition, when used in combination, tube growth also increased. Obviously, this was because of synergism between CCC and  $GA_3$ . We suggest



that CCC and GA<sub>3</sub> are acting at the same biochemical site resulting in the enhanced growth of the pollen tubes.

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**SAGRA FEMORATA DURRAY (SAGRINAE :  
CHRYSMELIDAE: COLEOPTERA) A NEW  
INSECT PEST OF DOLICHOS LABLAB**

GRUBS of a chrysomelid beetle, *Sagra femorata* Durray were found extensively damaging the country bean plants (*Dolichos lablab*) during July–August, 1972 at the Horticultural Experimental Farm, Hesaraghatta, of the Indian Institute of Horticultural Research. The grubs feed on the internal tissues of the stem (Fig. 1) and form the galls (Fig. 2) at the site of feeding. The



FIG. 1. L.S. of damaged portion of country bean stem showing the feeding of the grubs of *S. femorata*.

infested branches wilt and die. Several galls were found on each plant, each gall containing 3–4 grubs. Practically all the country bean plants cultivated over 0.25 ha area were damaged by the pest. Adults are metallic green and measure 2.4–2.6 mm in length and 0.95–1.05 mm in breadth. Eggs are laid on the stem. The larvae, after hatching, enter the stem and feed on the internal tissues without migrating to the adjacent stem portions. The full grown grubs pupate within the stem in an oval straw coloured cocoon made from chewed stem wood. Pupal stage lasts for 5–6 months. The pest has only one generation in a year. Similar damage was earlier reported on country bean<sup>2</sup> due to *Sagra nigrata* Oliver. The adults of *S. nigrata* are blue-black and are smaller in size than that of *S. femorata*.<sup>1</sup>



FIG. 2. Galls of the country bean stem formed by the feeding of *S. femorata*.

The authors are grateful to Dr. G. S. Randhawa, Director, for encouragement and to Mr. E. A. Duffy, Coleopterist of British Museum, London, for identification of the insect.

Indian Institute of Horticultural Research, Bangalore-560006, May, 9, 1975.

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### DISCOVERY OF PELECYPODS FROM THE CAMBRIAN ROCKS OF HIMALAYA

THICK successions of marine fossiliferous Cambrian rocks are exposed at various places of the Himalaya and adjoining areas including Kashmir, Ladakh, Spiti, Salt Range, etc. These successions are mainly known for the occurrence of certain well preserved trilobites, inarticulate brachiopods gastropods in them.

The fossiliferous rocks of the Cambrian System exposed in the Hundwara basin (Kashmir) which lies to the northwest of Central Crystalline axis were recently divided into Lower, Middle and Upper Series on the basis of fresh fossil finds<sup>1</sup>. The present paper, for the first time, places on record, the occurrence of pelecypods in the Middle Cambrian rocks of this part of the Kashmir valley. The occurrence of pelecypods was hitherto unknown from the Cambrian strata of Indian subcontinent. Even from other parts of the world, true Cambrian pelecypods are very rare.

The pelecypod material described in this communication was collected by the author while carrying out palaeontological and stratigraphical investigations of the Middle Cambrian beds exposed about 5 km northwest of Hundwara town. The specimens were collected in association with certain trilobites like *Tonkinella breviceps* Kobayashi, *Elrathina*? sp. *Obolus kashmiricus*, *Obolus* spp., *Lingulella* spp., *Lingulepis* sp., etc. The generic and specific identification of pelecypod specimens could not be carried out on account of paucity of well preserved material.

The beds yielding the pelecypods comprise shales, siltstones and sandstones of buff colour. They are highly jointed and the joints generally run oblique to the bedding plane.

The couple of pelecypod valves described below are catalogued in the collections of Centre of Advanced Study in Geology, P.U., Chandigarh, bearing numbers F838 and F839.

#### Description of Fossils

The two left valves of the pelecypods (one exterior—Fig. 1 and the other interior—Fig. 2) are almost similar in their morphological characters. Each valve in inequilateral with blunt beak, moderately convex transversely; umbo slightly swollen and anterior, the posterior part of the valve longer than anterior. In one of the valves (Fig. 1), fine, closely spaced concentric growth lines ornament the surface. In this valve, the lunule is poorly preserved whereas the escutcheon is well marked as compared to the other valve (Fig. 2) in which latter (escutcheon) is not as conspicuous as lunule. But this variation seems to be preservational one and not structural. The two valves are also almost comparable in their length-breadth ratio as follows:

#### Specimen F838 (Fig. 1):

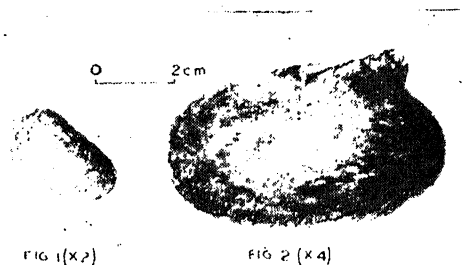
Length of the valve : 13.50 mm.  
Breadth of the valve : 8.00 mm.

#### Specimen F839 (Fig. 2):

Length of the valve : 14.00 mm.  
Breadth of the valve : 8.00 mm.

#### Comparison of Fossil Material

The surface ornamentation of the exterior of left valve (Fig. 1) is comparable to some extent with *Fordilla troyensis* Barrande known from the Lower Cambrian beds of New York State, Newfoundland, Greenland, England, Denmark and Portugal<sup>2</sup> and considered to be the oldest known pelecypod but the two strictly differ in their general outline. The outline of the present specimens is comparable with that of *Glyptarca primaeva* known from the Lower Ordovician rocks of South Wales, England (*vide* Schrock and Twerhofel, 1952, p. 401, Figs. 10-34), but the Kashmir specimens differ from the same in their size. The English form also possesses a different type of surface ornamentation. The problematic genus *Stenothecoides* Resser known from the upper Lower Cambrian to upper Middle Cambrian rocks of North America has a suboval to subelliptical elongate outline with apex overhanging unlike the Kashmir forms.



FIGS. 1-2.

#### Remarks

The two pelecypod valves recorded from Kashmir very slightly differ from each other in their overall shapes but only on this basis, the author feels that they cannot be attributed to two different species. They most probably represent two different individuals (both being left valves) belonging to same species.

The author is indebted to Dr. V. J. Gupta; for providing his collection of literature on the subject. He is also grateful to Dr. S. R. K. Chopra, for the facilities provided.

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## SHORT SCIENTIFIC NOTES

## A New Spot Test for Palladium Using Azine Dyes

Some of the spot tests for palladium are based on the accelerating effect of palladium on the reduction of: (1) phosphomolybdic acid by carbon monoxide<sup>1</sup>, (2) aqueous solutions of methyl orange, methylene blue, indigo carmine and malachite green by molecular hydrogen<sup>2</sup>, and (3) aqueous solutions of triphenylmethane, thiazine and azo dyes<sup>3</sup>.

This paper describes the use of six azine dyes, Phenosafranin, Methylene Violet, Amethyst Violet, Safranin, Wool Fast Blue BL (Colour Index Nos. 50200, 50210, 50225, 50240 and 50315 respectively) and Aposafrafin for the spot test detection of palladium.

0.01% solutions of the dyes were prepared in doubly distilled water. The dyes, Phenosafranin, Methylene Violet, Amethyst Violet, Safranin, and Aposafrafin used in this investigation were Gurr samples and Wool Fast Blue BL was a gift sample from M/s. Bayer AG, Leverkusen, Germany. The six dyes were employed without further purification. A stock solution (0.1%) of palladium chloride was prepared by dissolving palladium chloride (Johnson and Matthey sample) in water. This solution was suitably diluted with water. A saturated solution of sodium hypophosphite was prepared from May and Baker reagent grade sample. All other chemicals used were of reagent grade.

**Recommended Procedure.**—0.8 to 0.9 ml of saturated solution of sodium hypophosphite is taken in a micro test-tube and treated with one drop (0.05 ml) of 0.01% solution of Phenosafranin, Methylene Violet, Amethyst Violet, Safranin, Wool Fast Blue BL or Aposafrafin (0.15 ml in the case of Wool Fast Blue BL). One drop (0.05 ml) of the test solution is added and the mixture shaken well. Disappearance of the pink colour (blue in the case of Wool Fast Blue BL) indicates the presence of palladium. When the test is carried out at 96° C, still lower amounts of palladium can be detected. The identification and dilution limits at 28° C are 0.15 µg/ml and  $1:6.6 \times 10^6$  while the corresponding values at 96° C are 0.04 and  $1:2.5 \times 10^7$  respectively.

The following ions do not interfere in the test (the amounts are given in mg): Co (II): 0.5, Ni (II): 0.5, Pb (II): 0.03, Mn (II): 1.0, Ru (III): 0.035, Ag (I): 0.0016, Cu (II): 0.002, U (VI): 0.5, Au (III): 0.035, Pt (IV): 0.0025, Os (VIII): 0.015, Cl<sup>-</sup>: 0.5, Br<sup>-</sup>: 5, I<sup>-</sup>: 0.05, SO<sub>4</sub><sup>2-</sup>: 0.08, IO<sub>3</sub><sup>-</sup>: 0.08, S<sub>2</sub>O<sub>8</sub><sup>2-</sup>: 0.04, HSO<sub>4</sub><sup>-</sup>: 2, ClO<sub>4</sub><sup>-</sup>: 2, PO<sub>4</sub><sup>3-</sup>: 2, oxalate: 2, and tartrate: 2.

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Dothiorella Stem Canker on *Acacia mearnsii* Dewild

Large scale mortality of wattle (*Acacia mearnsii* Dewild), a plant cultivated for its tannin-rich bark was noticed in the Lower Pulneys since 1973. Brownish discolouration appeared initially at the place of branch initiation which spread, coalesce, encircle the stem, causing the shortening of the internodes, flattening of branch, development of splits, cracks and finally dieback of twigs and branches above the discoloured area. New shoots are formed by the activation of axillary buds near the seat of infection presenting a "witches' broom" appearance. Cankorous growth or hyperplasia leading to peculiar characteristic bulges also occur at branch junctions. The infected main stem 15–30 cm above the ground level turns black, gets flattened with longitudinal cracks, finally resulting in death of the plant. Isolations from the diseased twigs and branches yielded a dark coloured pathogenic fungus which on inoculation on healthy branches reproduced the disease symptoms within 10–12 days.

Irregular, dark, black, hard, carbonaceous stroma in masses, containing numerous pycnidia, arranged in one or more rows on the outer margins developed in month old culture of the pathogen. The pycnidia are more or less elliptic, oval to flask shaped, with or without an ostiole and measured  $244\text{--}430 \times 192\text{--}352 \mu$ . Single celled, slightly curved, hyaline, elongated, granulated pycnosporos with neither blunt nor pointed ends and measuring  $6 \text{ to } 10.6 \mu \times 2\text{--}2.5 \mu$ , developed on short, hyaline, single-celled pycnosporos. The fungus was identified as *Dothiorella pithyophilla* Sacc. et Penz, reported on pines<sup>1</sup>. However its infectivity on pines has not been tested. *Dothiorella mahagoni* Thum causes dark stem rot of *Swietenia mahagoni* (L.) Jacq. in West Indies and India<sup>2</sup> and *D. mangiferae* Syd dieback of *Mangifera indica* L. in India<sup>3</sup> and *D. populnea* Thum stem canker on *Populus tremuloides* Michx<sup>4</sup>. No record of this fungus as the causal agent of stem canker and dieback of wattle is available.

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#### *Aspergillus lanosus*—A New Mold Producing Citric Acid

Microbial production of citric acid has engaged the attention of several investigators owing to its apparent commercial potentialities. A project was undertaken for improving the yield of citric acid by screening of aspergilli from different substrates, by strain selection, and by induced mutation. Out of 70 *Aspergillus* species isolated and screened only 7 were found to possess the capacity to ferment commercial sugar into citric acid. This note reports the ability of *A. lanosus* Kam. et Bharg.<sup>1</sup> to bring about such a fermentation.

Microbial organic acid production was achieved by paper culture technique<sup>2</sup>. The production of citric acid was confirmed colorimetrically.<sup>3</sup> The stock culture of the fungus was maintained on Czapek's Agar slant from where it was inoculated into seed medium (Sugar 14%,  $\text{NH}_4\text{NO}_3$  0.25%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.025%,  $\text{KH}_2\text{PO}_4$  0.10%, soluble starch 0.10%, pH finally adjusted to 2.5 with N.HCl); 48 hours after incubation in seed medium, 5 ml of the medium containing the fungus was inoculated into production medium (Sugar 10%,  $\text{NH}_4\text{NO}_3$  0.18%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.018%,  $\text{KH}_2\text{PO}_4$  0.076%, pH finally adjusted to 2.5 with N.HCl). The cultures were incubated at 30°C for 7 days at 280 rpm.

After incubation the medium was filtered and the filtrate assayed for citric acid. The mycelium was washed and dried to determine the dry weight. The production of citric acid was estimated on the basis of sugar consumed by the mold. The concentrations of both the sugar and the acid in the culture medium were determined colorimetrically<sup>3,4</sup>, the latter by the method of Marier and Boulet<sup>4</sup>.

The mycelial dry weight was 3.8994 g/100 ml of the medium. The amount of sugar consumed in the

production medium was 5.50 g/100 ml while the citric acid yield was only 11.3 mg/ml of the medium. The rate of conversion of sugar into acid by *Aspergillus lanosus* is lower than that by several strains of *A. niger*. Attempts to increase the yield of citric acid by changing the factors governing its productivity are in progress.

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#### Fungi Associated with Sunflower Seeds

Two hundred seeds of each of the five commercially recommended varieties of sunflower, viz., EC. 68413, EC. 68414, EC. 68415, EC. 69874 and Sunrise were subjected to isolation procedure to know the association of various fungi. Both external and internal seedborne infections were detected by plating unsterilized and surface sterilized seeds on Blotter and Potato Dextrose Agar medium separately.

Twenty-two different fungal isolates were obtained as endo and ectophytic association with the seed. These isolates were identified as *Aspergillus flavus* Link ex Fries, *Aspergillus tamaritii* Kita, *Aspergillus niger* Van Tieghem, *Chaetomium globosum* Kunze ex Fr., *Neurospora* sp., *Curvularia lunata* (Wakker) Boedijn var. *aeria* (Batista, Lime and Vasconcelos) M. B. Ellis, *Macrophomina phaseolina* (Tassi) Goid., *Phoma exigua* Desm., *Aspergillus variegatus* var. *stellatus* Fennell and Raper, *Fusarium aquiseti* (Corda) Sacc., *Rhizopus microsporus* Van Tieghem, *Aspergillus sydowii* (Bainier and Sartory) Thom and Church, *Cladosporium* sp., *Alternaria* state of *Pleospora infectoria* Fuckel, *Aspergillus amstelodami* Thom and Church, *Verticillium* sp., *Drechslera hawaiiensis* M.B. Ellis, *Penicillium funiculosum*, *Fusarium moniliforme* Sheld and *Nigrospora sphaerica* (Sacc.) Mason. \*Two isolates did not sporulate and hence their identity could not be ascertained.

Out of these various fungal isolates *Aspergillus niger*, *Rhizopus microsporus*, *Aspergillus flavus*, *Macrophomina phaseolina* and *Aspergillus tamaritii* were found to be most frequently associated with the seeds. Pathogenicity tests revealed 26 to 30% loss in germination by these fungi.

The author thanks the Project Co-ordinator (Oilseeds) I.C.A.R., New Delhi, for providing facilities. Thanks are also due to C.M.I. for identification of isolates. Sunflower Research Scheme, J. G. RAUT. Punjabrao Krishi Vidyapeeth, Akola (M.S.), India, April 6, 1975.

#### A Preliminary Note on the Free amino Acids in *Centella asiatica* Linn.

*Centella asiatica* Linn. Urban Syn., *Hydrocotyle asiatica* Linn. (Umbelliferae) is a prostrate perennial, mildly aromatic herb found throughout India. This plant is widely used in Indian systems of medicine<sup>1,2</sup>. The present study is aimed at the detection and distribution of the free amino acids present in the different regions of the plant. The presence of five amino acids in this plant has been reported earlier.<sup>3</sup>

The plants for investigation were collected around Tiruchy town and separated into leaves, petioles, stolons and roots. A known quantity of each part was stabilized in 80% ethyl alcohol and the amino acid fraction extracted and identified by two-dimensional descending paper chromatography<sup>4</sup>.

Twenty free amino acids were identified in all the four different regions of the plant. They are : cysteine, cystine, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, arginine, lysine, histidine, tyrosine, amino-butyric acid, valine, methionine, proline, isoleucine, leucine, phenylalanine and tryptophan.

A colorimetric comparison of the quantity of the amino acids present in the different regions of the plant was made on the basis of the intensity on ninhydrin colour spot. It was found that the distribution of free amino acids in the leaves, petioles and stolons was about the same. In these regions glutamic acid, serine and alanine were found in larger quantities than the other amino acids. In the root, the various amino acids were found in greater quantities than in the other parts ; and in particular, aspartic acid, glutamic acid, serine, threonine, alanine, lysine, histidine and amino-butyric acid were in abundance.

Department of Botany, V. K. GEORGE.  
St. Joseph's College, J. L. GNANARETHINAM,  
Tiruchirappalli-620002, June 10, 1975.

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## REVIEWS AND NOTICES OF BOOKS

**World Meteorological Organization.** Technical Note No. 131. *Climate under Glass*. By Dr. J. Seemann. 1974. Pp. 40. Price not given.

Dr. J. Seemann has rendered a great service to agricultural scientists interested in raising tropical or sub-tropical crops in temperate or frigid zones under the suitably controlled environment of a glass-house. He has presented in this brochure a critical digest of several experiments in glass-houses already carried out under European conditions by workers in this area.

Stressing that solar radiation is a major factor, the author emphasises that in northern latitudes, the diffuse sky radiation can at times compete with the contribution from direct solar radiation. The glass or other plastic material enclosing the glass-house cuts off the ultra-violet and allows only a part of the solar energy to be transmitted into the interior of the glass-house. The blanketing effect of the glass or plastic covering as well as the energy balance at

the soil surface within the glass-house irradiated by solar radiations that reach that surface are greatly responsible for making the "climate under the glass" so much warmer than outside.

The author develops the subject under two main headings, viz., Chapter 1: "Elements and factors of climate in the greenhouse" and Chapter 2: "Control of climate in the greenhouse". In Chapter 1, the author discusses the several environmental factors like: (i) Radiation and heat balance, (ii) Heat transformation, (iii) Temperature conditions including air, soil and plant temperatures, (iv) Air humidity, (v) Evaporation and consumption of water and (vi) the Carbon-di-oxide factor. Chapter 2 covers topics like the basis of climatic control, regulation of temperature, shading, ventilation and water atomising installations and short period spraying.

The author has presented a clear picture of the large number of factors controlling the climate within the glass-house which is rendered congenial to the growth

and development of plants which would have no chance of survival under the extreme cold of the open climate. The various interesting points discussed are aptly illustrated by diagrams presenting the results of some of the glass-house investigations already carried out in Europe.

The brochure provides a list of references and bibliography on "Climate under glass".

We warmly recommend this valuable publication of W.M.O. for perusal by our agricultural scientists in India who may wish to utilise these techniques particularly in the northern regions of the country where crops and orchards are exposed to frost hazards in winter.

L. A. RAMDAS.

### Coping with Increasing Complexity—Implications of General Semantics and General Systems Theory.

By Donald E. Washburn and Dennis R. Smith. (Gordon and Breach, Science Publishers, New York London, Paris), 1974. Pp. x + 398. Price \$ 12.80.

Apart from the noise factors mathematically explored by information theorists, there is, in human communication, a much more fundamental source of distortion, attributable to the human mind itself and its conditioned 'world view'.

In recent times, starting from the beginning of this century, knowledge (specialized to a large extent) has been increasing at an explosively high rate. This has led to over-specialization and isolation of scientists in different disciplines and to the growth of mutually unintelligible jargons.

It is here the book under review comes to throw some light on the reorientation of the human mind, human attitudes and human linguistic and thinking habits, towards the search for *relations* and 'relations of relations', that is, towards the search for *isomorphism*. Nothing is absolute and permanent. All knowledge is relative.

Man (the observer), the human nervous system and the way in which the human mind psychologically, logically and linguistically dissects and 'maps' reality in terms of the symbols of his description, are all inalienably linked with the confusion of man's interaction with his ecology, ever increasingly becoming complex.

In the book, while the 'General Semanticist' is concerned with the structural similarities of language and the world it attempts to 'map', the 'General Systems Theorist' strives to discover structural similarities in different fields of knowledge. The book (an outgrowth of the joint conference sponsored by the Institute of General Semantics and the Society for General Systems Research) must be read by every educated thinking person, at least to be aware of the

limitations of our knowledge, the limitations of our logic and the limitations of our symbolic communications systems (the 'maps' like natural language (P. C. Ganeshsundaram, "Structural Relativity in Languages," paper presented to the 4th International Congress of Applied Linguistics, AILA, Stuttgart, August, 1975, to be published in the *Journal of the Indian Institute of Science*, Bangalore, in press, mathematical symbolism, etc.) and the limitations imposed by our own nervous system in 'mapping' the 'territory' of the external 'reality', which we can only interpret *relative* to ourselves and never in absolute terms.

Contributions to this book have been made by 28 scholars, some of whom are 'general semanticists' and some 'general systems theorists'.

P. C. GANESHSUNDARAM.

*Annual Review of Physiology* (Vol. 37), 1975. (Annual Reviews, Inc., Palo Alto, California 94306, U.S.A.). Pp. 558. Price not given.

The main aim of this *Annual Review* has been "to play a distinctive and useful role in providing the reader with a comprehensive survey of research in selected areas of physiology". This has been fairly achieved by the contributions which cover a wide range of topics. "Vestibular mechanisms" deal with the mechanisms of hair cell action and the physiology of the Vestibular end organs. "Electrophysiology of Neuroglia" brings home the conflicting opinions pertaining to the functions of the neuroglia; the properties of receptor units in the Somatosensory system and their Central Nervous System connections, "The Neural control of the pituitary" and "Structure function relationships in excitable membranes", the extra visual effects of visible and ultra-violet light on humans and other mammals and mechanisms involved in the control of body temperature are presented in various chapters. "Renal physiology" has been limited to dynamics of glomerular filtration, control of sodium reabsorption and uric acid excretion. Nervous and hormonal control mechanisms of vascular beds of the heart, brain, kidney and other organs is emphasised in the review on "Regional blood flow".

Advances in hormone research is dealt with in the reviews on "Hormonal regulation of the reproductive tract in female mammals", "the regulation of growth by endocrines" "Peripheral actions of glucocorticoids", "Circulating gastrin" and "erythropoietin". Physiology of respiration covers 'regulation of respiration in man' and 'defense mechanisms of the lungs'.

Of general interest are the topics on "Avian physiology", "Comparative physiology of Suspension feeding" and "The Sodium pump". M. SIRSI.

**Grape Varieties in India—Description and Classification.** By K. L. Chadha and G. S. Randhawa, (Indian Council of Agricultural Research, New Delhi), 1974. Pp. 221 52 Text-Figures. Price Rs. 10-25.

This is an attempt by the authors to describe the characters of 130 cultivated varieties of grapes in India. Most of these varieties are confined to a few research stations under the Indian Council of Agricultural Research. Nevertheless, the authors have given detailed descriptions of each variety, some of them illustrated with text-figures.

There are four Chapters, I. Introduction, II. Characters Used in Grape Description and Classification, III. Description of Varieties and IV. Classification of Grape Varieties. Chapter I is precise and brief. Chapter II reflects the thorough understanding of this specialized branch by the authors. The characters taken up for description and classification are well described and illustrated. Chapter III is descriptive, though somewhat repetitive, which perhaps could not be avoided in such a treatise. Chapter IV gives the classification of 136 varieties of grapes, with a key for identifying the varieties. A pictorial analysis of the grape varieties is also given.

In India we grow only a limited number of grape varieties. The fact that 130 varieties are available in the research stations in India, for economic exploitation by the farmers is clear from the data given in the book. Considering there are about 7,000 varieties of grapes in the world, this number of 130 is too small. However, at least some of the more promising varieties under Indian conditions should be taken up for wider cultivation. The authors' attempt to bring out the various characters of these varieties would stand credited if our extension workers take the lead from this point. The key for classification of grape varieties given by the authors is rather complicated, though the authors seem to feel that the key is "simple and suitable for rapid and accurate identification of varieties". Considering that several of these characters, particularly the colour of the fruits and of the leaves vary perceptibly under the influence of different climatic factors and the soil and other agronomic factors, including nutritional deficiencies in the soil, some of the characters suggested for classification and identification may not be very accurate. What is applicable to Delhi conditions may not be applicable to Bangalore, or Dindigul condition in all the varieties. Perhaps with additional information collected over a period of years, from different agro-climatic regions of the country, the key may be suitably modified.

The authors' efforts to collect all the available information on grape varieties in India, and present them

in a systematic and meaningful manner are highly commendable. This monograph will be an asset to the research workers and students specialising in grapes. Extension workers and progressive farmers could get rich information on the scope for grape cultivation of a widely varying number of varieties under Indian conditions. The book could be a valuable addition to the libraries in India.

G. RANGASWAMI,

### **Magnetic Resonance in Chemistry and Biology.**

Ed. Janko N. Herak and Kresimir J. Adamic, (Marcel Dekker, Inc., New York), 1975. Pp. ix + 551, Price \$ 34.50.

This book is a collection of articles based on lectures at the Ampere International Summer School on Magnetic Resonance, held in Yugoslavia in 1971. The stated purpose of this book is to 'introduce magnetic resonance to younger scientists working in those natural sciences for which these techniques have proven particularly useful'. The chapters vary widely in their level of sophistication and their usefulness to chemists and biologists. The introductory articles on Spectroscopy (Adamic), NMR (Bovey) and ESR (Bard) may prove useful to the uninitiated. The articles on Applications of Magic Angle Rotation, Study of Molecular Motion in Solids and Spin-Lattice Relaxation in Low Magnetic Fields are perhaps unnecessary for the non-spectroscopist. The chapter on Mathematical Analysis of Distortion Effects in an ESR Spectrometer and the discussion of Signal to Noise Ratio of NMR Oscillator Detectors are of interest only to a small fraction of 'natural scientists', who are instrumentally minded, while the last two chapters dealing with Dielectric Measurements are totally out of place.

Chemically, interesting applications of ESR to kinetic problems and electrochemistry are described and may prove useful. The biologically useful chapters are those dealing with 'Radiation Induced Processes in Nucleic Acids' (Herak), 'Hemoglobin' (Maricic) and the description of Studies of atomic and molecular motions by NMR (Jones). The article on High Resolution NMR of biopolymers is too brief to be of much use to the non-specialist. The book has been published four years after the conference and does in no way accomplish its stated purpose of introducing magnetic resonance to 'natural scientists'. The publication of conference proceedings of this type serves only to enhance the list of publications of the contributing authors. It is time the scientific community took a serious look at the duplication of reviews and papers to prevent our libraries from being drowned in a sea of redundant material.

P. BALARAM,

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# BETA-GAMMA DIRECTIONAL CORRELATION MEASUREMENT IN $^{194}\text{Ir}$

D. K. PRIYADARSINI, B. VEMA REDDY AND D. L. SASTRY

Laboratories for Nuclear Research, Andhra University, Waltair

## ABSTRACT

The angular correlation of the  $1^- \xrightarrow[1920 \text{ keV}]{\beta} 2^+ \xrightarrow[329 \text{ keV}]{\gamma} 0^+$  cascade is measured integrally and at two beta energies. The results show isotropic correlation and indicate the validity of the  $\xi$  or coulomb approximation for the 1920 keV beta transition of  $^{194}\text{Ir}$ .

## INTRODUCTION

THE odd-odd iridium nucleus has 77 protons and 117 neutrons, lying between highly deformed and nearly doubly magic spherical nuclei. It decays with half life 17 hrs to the even-even  $^{194}\text{Pt}$  via beta decay populating a number of positive parity states. The ground state of  $^{194}\text{Ir}$  has  $1^-$  character and the following beta transitions to  $^{194}\text{Pt}$  are thus of non-unique first forbidden type. Information on beta decay observables and the concerned matrix elements in this region will be of great importance from a theoretical point of view as several approaches have been suggested to describe the nuclear levels. And, in particular, the Nilsson model and the quasi-particle description of the nuclear states have been tried to explain the various experimental results.

A partial decay scheme of  $^{194}\text{Ir}$  of present interest and as taken from Ref. 1 is shown in Fig. 1. The  $1^- \xrightarrow[1920 \text{ keV}]{\beta} 2^+ \xrightarrow[329 \text{ keV}]{\gamma} 0^+$  cascade is of present interest. The

1.92 MeV beta component from  $^{194}\text{Ir}$  feeding the first excited state of  $^{194}\text{Pt}$  with 329 keV energy has an intensity of 5.1% and log ft value 9.2. The  $\xi$ -value for this is 10.04 and is much greater than  $W_0 - 1$  ( $= 3.758$ ), where  $W_0$  is the end-point energy expressed in  $m_e c^2$ . Thus the  $\xi$ -value is consistent with the number expected for high Z nuclei. A study of beta-gamma anisotropy will be useful to test the validity of  $\xi$ -approximation. There was only one earlier measurement on the shape and angular correlation concerning the 1.92 MeV beta transition by Deutsch *et al*<sup>2</sup>. They report a statistical shape and isotropic angular correlation consistent with the validity of  $\xi$ -approximation. In the present study a reinvestigation of the beta-gamma angular correlation

of the  $1^- \xrightarrow[1920 \text{ keV}]{\beta} 2^+ \xrightarrow[329 \text{ keV}]{\gamma} 0^+$  cascade is considered to confirm the earlier measurement and the results are discussed for the applicability of  $\xi$ -approximation.

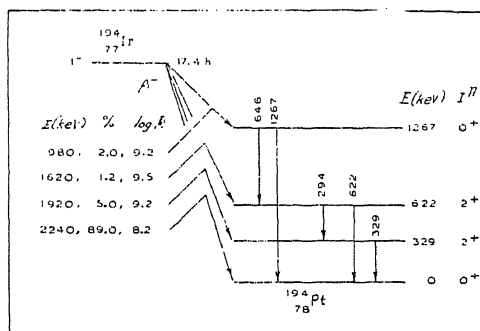
## EXPERIMENT AND RESULTS

The experiment is carried out on a conventional slow-fast scintillation assembly, associated with a two-channel arrangement. A  $1\frac{1}{2}'' \times 1''$  NaI (Tl)

crystal optically coupled to a RCA 6810-A photo-multiplier accomplishes gamma detection. A conical lead shield houses the gamma crystal for collimation of gamma radiation. The beta detector is a plastic scintillator with conical well cut in it to reduce low energy tailing arising out of backscattering effects. The source, being situated at the apex of the conical well, the effective solid angle is about 2% of  $4\pi$ .

$^{194}\text{Ir}$  source was obtained in liquid form as Sodium iridate in HCl from Bhabha Atomic Research Centre, Bombay (India). The source for the present measurements was prepared on a mylar film of thickness  $0.6 \text{ mg/cm}^2$  over an area of 3 mm, the source film being glued to a very thin aluminium ring of diameter one inch.

*Integral correlation.*—From Fig. 1 it may be noted that only betas of energy above 1.6 MeV cascading



Decay scheme of  $\text{Ir-}^{194}$

FIG. 1

with the 329 keV gammas are free of interferences from the other cascades. At this energy as the intensity will be low, first the integral correlation experiment was performed to have an idea of the beta-gamma anisotropy. In this, betas of energy 1.6 MeV and above were accepted in the beta channel while the 329 keV gammas were accepted in the gate as indicated in Fig. 2. The coincidence data were collected at three angles  $90^\circ$ ,  $135^\circ$  and  $180^\circ$  to compensate for the short half life 17 hrs of  $^{194}\text{Ir}$ . The pooled up coincidences are corrected

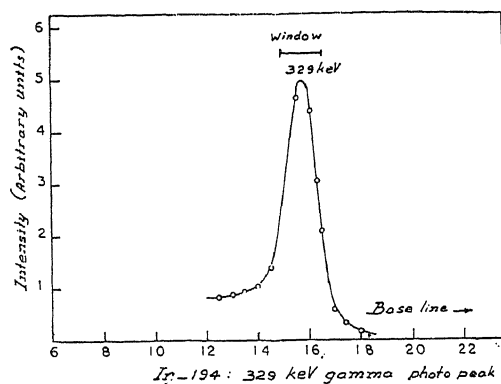


FIG. 2

for chance and background and normalised to the corresponding singles counts from the movable gamma detector. After applying the geometrical corrections to  $A_{22}$  and  $A_{44}$ , the following  $\beta$ - $\gamma$  angular correlation function is obtained, by employing White's formulae<sup>3</sup>.

$$W(\theta) = 1 + (0.003 \pm 0.01) P_2(\cos \theta) \\ + (0.005 \pm 0.0397) P_4(\cos \theta)$$

The integral correlation results are shown in Fig. 3 as a function of  $\theta$ . The straight line nature of the plot shows the absence of the  $A_{44}$  coefficient in the angular correlation function, thus establishing the non-unique first forbidden nature of the 1.92 MeV beta transition in  $^{194}\text{Ir}$ .

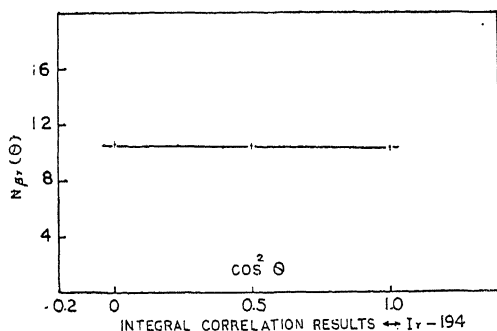


FIG. 3

**Differential correlation.**—These measurements were performed at two beta energies 4.22 and 4.351 (in  $m_0c^2$  units) in a window of 75 keV. The coincidences were collected at  $90^\circ$  and  $180^\circ$  and all the corrections were applied to the observed coincidences to get the true coincidences. The following  $A_{22}$  coefficients are obtained at the two

beta energies after applying the geometrical corrections.

| Beta energy in $m_0c^2$ units | Correlation coefficient |
|-------------------------------|-------------------------|
| 4.22 (=1.6375 Mev)            | $0.005 \pm 0.009$       |
| 4.351 (=1.7125 MeV)           | $0.002 \pm 0.009$       |

The results of both integral and differential correlation clearly establish that the beta-gamma angular correlation is isotropic within experimental uncertainties, in conformity to the results reported by Deutsch *et al.*

## DISCUSSION

The large  $\xi$ -value and the isotropic angular correlation support the validity of the  $\xi$ - or coulomb approximation for the 1.92 MeV beta transition in  $^{194}\text{Ir}$ . The statistical shape of the same reported by Deutsch *et al.* confirms this conclusion. It is normally difficult to obtain nuclear matrix elements of beta transitions following  $\xi$ -approximation unless, the number of available experimental observables are large. And such an attempt could be successfully made only in the case of the 960 keV beta transition of  $^{198}\text{Au}$  (ref. 4) which follows the  $\xi$ -approximation. A similar attempt can also be made for the present beta transition if data on different types of polarisation are available. In the absence of it there is not much to say anything about the validity of model predicted matrix elements. However, Deutsch *et al.* make an attempt for the determination of the nuclear matrix element parameter ratios  $u/x$  and  $z/x$  using their experimental data on  $C(W)$  and  $\epsilon(W)$ . In this, they assumed the validity of the CVC relationship due to Fujita and employed the approximate formulas of Kotani<sup>5</sup>. However, in the light of Damgaard and Winther<sup>6</sup> hypothesis and Buhning's<sup>7</sup> formulae (with Simms<sup>8</sup> method of application), the attempt of Deutsch is only an approximation.

From the present measurements on beta-gamma directional correlation it may be concluded that the 1.92 MeV beta transition of  $^{194}\text{Ir}$  follows  $\xi$ -approximation.

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# EFFECT OF 2-MERCAPTOETHANOL ON HAEMAGGLUTININ-ERYTHROCYTE AND HAEMOLYSIN-ERYTHROCYTE COMPLEX

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## ABSTRACT

In rat and rabbit anti-sheep erythrocyte sera, in primary response, the haemolysin activity is associated with IgM whereas haemagglutinin activity is exhibited by both IgM and IgG. Erythrocyte-haemolysin complex, on treatment with 2-mercaptoethanol, loses its capacity to be lysed by complement, suggesting that the structural integrity of IgM is essential for complement action.

## INTRODUCTION

**S**HEEP erythrocytes are often used as standard antigen in immunological investigations. At humoral level, the haemolytic and haemagglutinating properties of anti-sheep erythrocyte antibodies are estimated and at cellular level, the number of plaque-forming cells<sup>1</sup> and rosette-forming cells<sup>2</sup> are measured.

Deutsch and Morton<sup>3</sup> first described that IgM antibody is converted into 7S monomers by treatment with 2-ME (0.1 M) whereas IgG is not affected. The reduced IgM monomers are shown to retain their capacity to bind with hapten<sup>4</sup>. In this work using 2-ME sensitivity and Sephadex G-200 gel filtration, the haemolytic anti-sheep erythrocyte antibody in peak primary response is found to belong to IgM class whereas haemagglutinating antibody belongs to both IgM and IgG classes. Further, erythrocyte-haemolysin complex when treated with 2-ME loses its capacity to be lysed by complement, indicating that structural integrity of IgM is necessary for complement action.

## MATERIALS AND METHODS

Wistar A/His rats were immunised by injecting  $3 \times 10^{10}$  SE i.p. Blood was collected, during peak response, on 7th day after immunisation. Serum was separated by centrifugation, inactivated at 56° C for 30 min, and stored frozen. Rabbit antiserum was obtained similarly by administering  $5 \times 10^{10}$  SE i.v. Haemagglutination titrations were carried out in 0.15 M NaCl and haemolysin titrations in modified barbital buffer as described by Campbell *et al.*<sup>5</sup>. Antiserum was fractionated on Sephadex G-200 gel with 0.15 M phosphate-saline buffer (pH 7.2). Three ml fractions were collected and  $A_{260}$  recorded.

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Abbreviations used: Sheep erythrocytes (SE); 2-mercaptoethanol (2-ME); modified barbital buffer (diluent).

## RESULTS AND DISCUSSION

The elution profile of rat anti-sheep erythrocyte serum is shown in Fig. 1. The haemagglutinin activity is located in two peaks, I and II, peak I being eluted with the exclusion volume of the column. The haemolytic antibody fraction is solely located in peak I. The haemolytic activity of the unfractionated antiserum as well as that of peak I was lost when incubated with 2-ME (0.1 M). Concomitantly, the haemagglutinin and haemolytic activity of the fractions in the region of peak I were lost. Under the experimental conditions employed, 2-ME is known to reduce the IgM to subunits<sup>3</sup>.

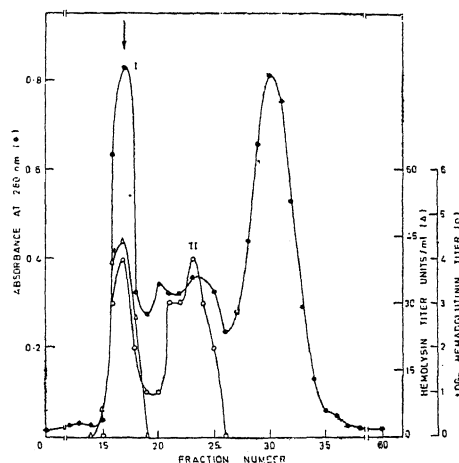


Fig. 1. Sephadex G-200 profile of rat anti-SE Serum (column  $2 \times 45$  cm).

Removal of 2-ME from the reaction mixture was not found necessary for haemagglutination test with anti-SE serum since these haemagglutinins are not affected by 2-ME, and consequently alkylation was not required. Natural heterohaemagglutinins also have been reported to behave in similar way<sup>6</sup>. However, no lysis of erythrocytes was observed when 2-ME was not removed from the reaction mixture of haemolysin

titration. This would mean that 2-ME is interfering with one or more of the following steps: (1) Modification of erythrocytes so that they are resistant to lysis by antibody in presence of complement. (2) Destruction of anti-body-erythrocyte complex. (3) Destruction of complement activity. The following experiments were performed to check these possibilities. Rat anti-SE serum was used in following experiments.

Sheep erythrocytes were incubated with varying concentrations of 2-ME (0.001 M, 0.01 M and 0.1 M). 2-ME was removed by centrifugation, and the pellet was washed and complexed with antibody. Haemolysis was observed, as in controls where sheep erythrocytes were not incubated with 2-ME, indicating that 2-ME does not affect the erythrocytes (Table I). Next, antibody-erythrocyte complex was incubated with 2-ME and the latter removed by centrifugation and washings. In this case

TABLE I  
*Effect of 2-ME on various components of haemolysin titration of rat anti-SE serum*

| Material treated        | % activity of haemolysin activity of antiserum |        |
|-------------------------|--|--------|
|                         | + 2-ME   | - 2-ME |
| (a) Antiserum           | 5-10   | 100    |
| (b) SE                  | 100  | 100    |
| (c) Antibody-SE complex | 25   | 100    |
| (d) Complement          | 40-45  | 100    |

(a) Anti-SE serum and 2-ME (0.1 M) were incubated at room temperature for 60 min followed by incubation with SE ( $3 \times 10^9$  cell/ml) at 37° C for 30 min. The tubes were centrifuged, the pellet was washed, re-suspended in diluent. After this, titrations were carried out as described in text.

(b) SE ( $3 \times 10^9$  cells/ml) were incubated with 2-ME (0.001 M, 0.01 M, and 0.1 M) at room temperature for 60 min. The tubes were centrifuged and erythrocytes washed with diluent. The pellet was suspended in diluent and incubated with anti-SE serum at 37° C for 30 min followed by complement. The titrations were carried out as described in text.

(c) The complex was formed by incubating anti-SE serum and SE ( $3 \times 10^9$  cells/ml) for 30 min at 37° C. The tubes were centrifuged and pellet washed with diluent. The pellet was resuspended in diluent and incubated with 2-ME (0.1 M) at room temperature for 60 min. The tubes were then centrifuged and washed. The pellet was resuspended in diluent and titrations were carried out as described in text.

(d) Complement was incubated with 2-ME (0.1 M) at room temperature for 60 min. 2-ME was removed by passing the mixture through G-15 column equilibrated with isotonic barbital buffer and by eluting with the same buffer. This complement was used for carrying out haemolysin titrations.

Suitable controls were included in every experiment.

inhibition was observed (Table I). Thus, there are two possibilities: (1) 2-ME reduces the antibody in the complex causing either dissociation of monomeric subunits of IgM from erythrocytes or insensitivity of the resulting monomer-erythrocyte complex to complement action and (2) 2-ME dissociates the IgM from complex and subsequently reduces it to monomeric subunits. We believe it is more logical that 2-ME reduces IgM in complex and the monomer-erythrocyte complex is insensitive to complement action. It has been shown that  $C_1$  complement fixation needs at least one IgM or two IgG molecules which would mean that initial 'polymeric' IgM structure is important for complement fixation<sup>7</sup>. IgM when treated with 2-ME also loses its  $C_1$  complement fixing activity<sup>7</sup>. The second possibility seems unlikely as no covalent linkages are involved in antigen-antibody complex; further, IgG-erythrocyte complex is not affected by 2-ME. However, if sulphhydryl functions are involved in IgM-erythrocyte complex formation and that is affected by 2-ME then dissociation of IgM from the complex and its subsequent reduction may result.

To check whether complement activity is affected by 0.1 M 2-ME, the complement was incubated with 2-ME (0.1 M) and filtered through Sephadex G-15 to remove unreacted 2-ME from the complement. This preparation when added to sensitized erythrocytes showed 40-45% inhibition of lysis as compared to the control preparation which was not subjected to 2-ME treatment (Table I). The sensitivity of complement to 2-ME necessitated the modification in haemolysin titration.

The effect of varying concentrations of 2-ME (0.001 M, 0.01 M and 0.1 M) and anti-SE serum (1/10, 1/100, 1/1000) was examined (Table II). From the results it appears that reduction of IgM can be achieved at lower concentrations of 2-ME if the concentration of antibody is also reduced.

TABLE II  
*Effect of varying concentrations of 2-ME on different concentrations of rat anti-SE serum*

| Concentration of 2-ME | Concentration of anti-SE serum | % Haemolysis of control (No. 2-ME) |
|-----------------------|--------------------------------|------------------------------------|
| 0.1 M                 | 1/10                           | 5-10                               |
| 0.01 M                | 1/10                           | 90                                 |
| 0.001 M               | 1/10                           | 90                                 |
| 0.01 M                | 1/100                          | 45                                 |
| 0.001 M               | 1/1000                         | 100                                |

Haemolysin titrations were carried out as described in Table I.

Similar results were obtained with rabbit anti-SE serum (Table III). Both in rat and rabbit, at the peak of primary response, haemolysis is due to IgM, and agglutination is exhibited by both IgG and IgM. These results indicate that 2-ME affects IgM both in free and complexed states.

TABLE III

Effect of 2-ME on various components of haemolysis titration of rabbit anti-SE serum

| Material treated        | % activity of haemolysis activity of antiserum |      |
|-------------------------|--|------|
|                         | 2-ME   | 2-ME |
| (a) Anti-SE serum       | 10   | 100  |
| (b) SE                  | 100  | 100  |
| (c) Antibody-SE complex | 25   | 100  |
| (d) Complement          | 40-45  | 100  |

a, b, c and d are same as in Table I.

## ACKNOWLEDGEMENT

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## DIELS-ALDER REACTION OF TETRACYCLONE WITH SOME MALEIMIDES

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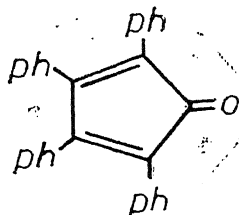
## ABSTRACT

Tetracyclone reacts with N-substituted maleimides II to give the adducts III a-f. Dehydrogenation of III c, e, f gave IV a-c. In a similar manner III adds another molecule of either maleic anhydride or N-substituted maleimide to give V a-b and VI a-e respectively. On the other hand, VI b dehydrogenates readily to VII.

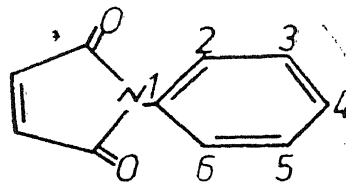
**T**ETRAPHENYLCYCLOPENTADIENONE (tetracyclone) I and its analogues were reported to undergo Diels-Alder reaction with ethylenic dienophiles<sup>1-13</sup>.

We now succeeded to isolate the adducts III a-f from the reaction of one molecule of N-substituted maleimides II a-f with one molecule of tetracyclone in bromobenzene or in dry toluene. III c-e are dehydrogenated readily with bromine to give IV a-c.

The structure assigned for the addition products III a-f has been supported by analytical and spectral (U.V., I.R. and N.M.R.) data. III e, for example, shows a carbonyl two bands widely separated at 1770 cm<sup>-1</sup> and 1690 cm<sup>-1</sup> (for -CO·NH·CO-)<sup>15</sup>. The U.V. spectrum of III c showed an absorption band at 340 mμ.<sup>16</sup> The structure of the adducts obtained has been further evidenced by the N.M.R. spectrum. For example the N.M.R. spectrum of III a showed



I



II

a, 2-COOH

b, 3-COOH

c, 4-COOH

d, 2-Me, 3-Cl

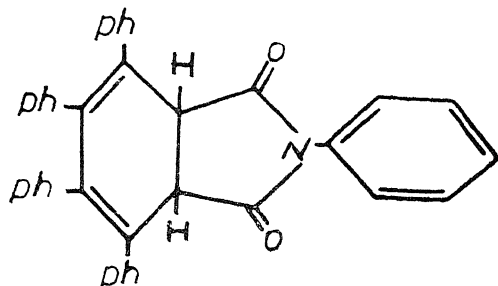
e, 2-Me, 5-Cl

f, 4-Me, 3-Cl

\* To whom all the correspondence should be addressed.

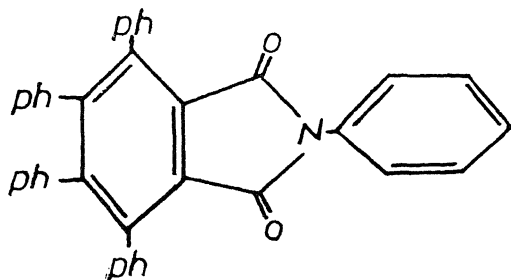
a two singlet at  $\delta$  4.4, 4.45 for the two hydrogen protons and a singlet at  $\delta$  2.15 for the methyl group. Moreover two multiplets at 6.8  $\delta$  and 7.1  $\delta$  has appeared for the aromatic protons.

Similarly the structures of IV *a, b* have been proved by analytical data and I.R. spectra. Thus the I.R. spectrum of IV *b* shows absorption at  $1750\text{ cm}^{-1}$  and  $1690\text{ cm}^{-1}$  (for  $-\text{CO}-\text{NH}-\text{CO}-$ )<sup>15</sup>. In addition, the structure of IV *c* has been confirmed by m.p. and mixed m.p. with an authentic sample obtained from the reaction of tetraphenylphthalic anhydride<sup>14</sup> with 4-methyl-3-chloroaniline followed by cyclization.



III

- a*, 2-COOH  
*b*, 3-COOH  
*c*, 4-COOH  
*d*, 2-Me, 3-Cl  
*e*, 2-Me, 5-Cl  
*f*, 4-Me, 3-Cl

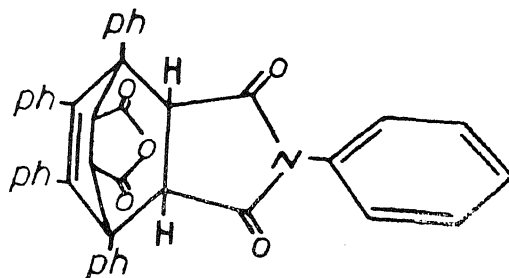


IV

- a*, 4 COOH  
*b*, 2 Me, 5-Cl  
*c*, 4 Me, 3-Cl

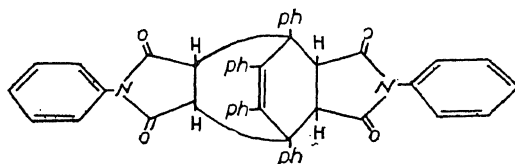
The appearance of an absorption band at  $340\text{ m}\mu$  in the U.V. spectrum<sup>16</sup> of III *c*, has been established chemically by the addition of another molecule of dienophile on the created diene. So III adds one molecule of maleic anhydride to give the endo-adducts V *a, b*. The structure of V has been proved from the analytical data, I.R. spectrum of V *a*, which shows absorption at  $1740\text{ cm}^{-1}$  and  $1700\text{ cm}^{-1}$ . On the other hand, III *a-f* add another molecule of N-substituted

maleimides to give an exo-adduct VI *a-e* on the diene formed. VI *b* is readily dehydrogenated to give VII.



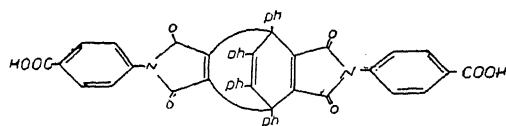
V

- a*, 3-COOH  
*b*, 2-Me, 3-Cl



VI

- a*, 3-COOH  
*b*, 4-COOH  
*c*, 2-Me, 3-Cl  
*d*, 2-Me, 5-Cl  
*e*, 4-Me, 3-Cl



VII

The structure of VI *a-e* and VII are established from the analytical data and I.R. spectra. The I.R. spectrum of VI *e* shows absorption band at  $1760\text{ cm}^{-1}$  and  $1690\text{ cm}^{-1}$ , and that of VII at  $1790\text{ cm}^{-1}$ , and  $1670\text{ cm}^{-1}$ <sup>15</sup>. Also the m.p. and mixed m.p. of VI *b* obtained from one or two steps confirms the above structure.

#### EXPERIMENTAL PROCEDURE

All the melting points are uncorrected. I.R. spectra are obtained on potassium bromide pellets, on a Perkin-Elmer spectrophotometer. U.V. spectra are obtained in ethanol, on a Beckman DK-2 spectrophotometer. The N.M.R. are obtained in  $\text{CDCl}_3$  solution using a tetramethylsilane as internal standard, on a Varian A-60.

**Action of tetracyclone on N-substituted maleimides.**—A mixture of I (1.9 g) and the appropriate N-substituted maleimide II (0.9 g) are refluxed in 20 ml of either bromobenzene or dry toluene for three hours,

TABLE I  
Analytical data of the adducts III a-f

| Adducts* | m.p.<br>°C | Mol. Formula  | C%<br>Found (Calc.) | H%<br>Found (Calc.) | N%<br>Found (Calc.) | Cl%<br>Found (Calc.) |
|----------|------------|---|---------------------|---------------------|---------------------|----------------------|
| III a    | 261        | C <sub>39</sub> H <sub>27</sub> N O <sub>4</sub>    | 81.32 (81.66)       | 4.72 (4.74)         | 2.39 (2.44)         | ..                   |
| III b    | 140        | C <sub>39</sub> H <sub>27</sub> N O <sub>4</sub>    | 81.51 (81.66)       | 4.70 (4.74)         | 2.36 (2.44)         | ..                   |
| III c    | 252        | C <sub>39</sub> H <sub>27</sub> N O <sub>4</sub>    | 81.49 (81.66)       | 4.75 (4.74)         | 2.39 (2.44)         | ..                   |
| III d    | 234        | C <sub>39</sub> H <sub>28</sub> N O <sub>2</sub> Cl | 81.02 (81.04)       | 4.81 (4.85)         | 2.38 (2.42)         | 6.16 (6.15)          |
| III e    | 356        | C <sub>39</sub> H <sub>28</sub> N O <sub>2</sub> Cl | 80.91 (81.04)       | 4.82 (4.85)         | 2.43 (2.42)         | 6.00 (6.15)          |
| III f    | 328        | C <sub>39</sub> H <sub>28</sub> N O <sub>2</sub> Cl | 81.12 (81.04)       | 4.79 (4.85)         | 2.40 (2.42)         | 6.11 (6.15)          |

\* III a, b, d are crystallized from ethanol; III c crystallized from benzene/benzine; III e crystallized from chloroform/benzine; III f crystallized from benzene/alcohol; the compounds are obtained in 58–70% yields

TABLE II  
Analytical data of the adducts VI a-e

| Adducts* | M.P.<br>°C | Mol. Formula  | C%<br>Found (Calc.) | H%<br>Found (Calc.) | N%<br>Found (Calc.) | Cl%<br>Found (Calc.) |
|----------|------------|---|---------------------|---------------------|---------------------|----------------------|
| VI a     | 301        | C <sub>50</sub> H <sub>31</sub> N <sub>2</sub> O <sub>8</sub>                 | 76.01 (75.94)       | 4.31 (4.33)         | 3.51 (3.54)         | ..                   |
| VI b     | 343        | C <sub>50</sub> H <sub>31</sub> N <sub>2</sub> O <sub>8</sub>                 | 75.86 (75.94)       | 4.30 (4.33)         | 3.53 (3.54)         | ..                   |
| VI c     | 265        | C <sub>50</sub> H <sub>36</sub> N <sub>2</sub> O <sub>4</sub> Cl <sub>2</sub> | 75.00 (75.10)       | 4.46 (4.50)         | 3.49 (3.50)         | 8.92 (8.90)          |
| VI d     | 233        | C <sub>50</sub> H <sub>36</sub> N <sub>2</sub> O <sub>4</sub> Cl <sub>2</sub> | 75.05 (75.10)       | 4.48 (4.50)         | 3.51 (3.50)         | 8.81 (8.90)          |
| VI e     | 248        | C <sub>50</sub> H <sub>36</sub> N <sub>2</sub> O <sub>4</sub> Cl <sub>2</sub> | 74.98 (75.10)       | 4.46 (4.50)         | 3.47 (3.50)         | 8.86 (8.90)          |

\* VI a crystallized from ethanol; VI b crystallized from dil. ethanol; VI d and VI e crystallized from benzene/alcohol; VI c crystallized from benzene/benzine. Compounds are obtained in 70–80% yields.

then allowed to cool. The solid products so formed are filtered and crystallized from the suitable solvent (*cf.* Table I) and identified as III a-f.

*Action of maleic anhydride on III b, d.*—A mixture of III b or III d (1.1 g) and maleic anhydride (0.2 g) are heated in 20 ml of either bromobenzene or dry toluene, the reaction mixture is refluxed for three hours, then allowed to cool. The solid products so obtained are filtered and crystallized from the suitable solvent and identified as V a, b.

V a crystallized from ethanol, mp. 175°C; yield 70% (Found: C, 77.01; H, 4.28; N, 2.03. Calcd. for C<sub>43</sub> H<sub>29</sub> N O<sub>7.5</sub>: C, 76.90; H, 4.32; N, 2.08).

V b crystallized from benzene/benzine; m.p. 244°C; yield 63% (Found: C, 76.28; H, 4.41; N, 2.06; Cl, 5.19. Calcd. for C<sub>43</sub> H<sub>30</sub> N O<sub>5</sub> Cl: C, 76.38; H, 4.44; N, 2.07; Cl, 5.26).

*Action of N-substituted maleimides on III a-f.*—A mixture of III b-f (1.1 g) and the appropriate N-substituted maleimide (0.9 g) are heated together in dry toluene (20 ml), the reaction mixture is refluxed for three hours and allowed to cool. The solid products are filtered and crystallized from the suitable solvent (*cf.* Table II) and identified as VI a-e.

*Dehydrogenation.*—The same procedure used for the synthesis of tetraphenylphthalic anhydride<sup>14</sup> has



been applied for the dehydrogenation of III *c*, *e*, *f* and VI *b* to give IV *a-c* and VII respectively.

IV *a* crystallized from ethanol, m.p. 359° C; yield 70% (Found: C, 81.92; H, 4.32; N, 2.41, Calcd. for  $C_{39}H_{25}NO_4$ : C, 81.96; H, 4.38; N, 2.45).

IV *b* crystallized from benzene/alcohol, m.p. 342° C; yield 70% (Found: C, 81.33; H, 4.49; N, 2.45; Cl, 6.13. Calcd. for  $C_{39}H_{26}N_2O_2Cl$ : C, 81.32; H, 4.52; N, 2.43; Cl, 6.17).

IV *c* crystallized from ethanol, m.p. 260° C; yield 73% (Found: C, 81.29; H, 4.51; N, 2.44; Cl, 6.15. Calcd. for  $C_{39}H_{26}N_2O_2Cl$ : C, 81.32; H, 4.52; N, 2.43; Cl, 6.17).

VII crystallized from dil. ethanol, m.p. 353° C; yield 70% (Found: C, 76.25; H, 3.76; N, 3.52. Calcd. for  $C_{50}H_{30}N_2O_8$ : C, 76.33; H, 3.84; N, 3.56).

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## OBITUARY

### PROFESSOR T. R. SESHADRI, F.R.S. (1900-1975)

**P**ROFESSOR Tiruvenkata Rajendra Seshadri was born on the 3rd February 1900 at Kulittalai in Tamil Nadu (South India). He received his school education at Srirangam and Tiruchirapalli. He studied at the Presidency College, Madras, for the Honours degree in Chemistry of the University of Madras. Subsequently he served the Ramakrishna Mission, Madras, for a year organising their newly started residential high school. Later he worked for 3 years at the Chemistry Department, Presidency College, Madras, under the guidance of Professor B. B. Dey, as a Madras University research scholar. His researches were on Indian Medicinal plants and on coumarins, and for these he received the Sir William Wedderburn and the Curzon prize of the University of Madras.

In 1927 with an overseas scholarship awarded by the Government of Madras, Mr. Seshadri joined the University of Manchester to work under Prof. Robert Robinson, F.R.S., one of the most distinguished organic chemists who ever lived, who later received the Knighthood and the Order of Merit of the United Kingdom and was President of the Royal Society and also a recipient of the Nobel Prize. When Prof. Robinson moved to University College, London, Mr. Seshadri also followed him. For his thesis entitled "Search for New Antimalarials and the Synthesis of Anthocyanins" the Manchester University awarded him the degree of Doctor of Philosophy in 1929. The entry into Robinson's school was cherished by Dr. Seshadri as the most important event of his research career.

After finishing his formal work for Ph.D., Dr. Seshadri worked for some months in the laboratory of Professor Fritz Pregl, Nobel Laureate, in Graz (Austria), famous as the home of organic micro-analysis. He also worked with Professor George Barger, F.R.S., at the Department of Medical Chemistry of the University of Edinburgh. He also spent some time in the laboratory of the Chief Agricultural Analyst to the county of Fife.

Dr. Seshadri returned to India in 1930 at a time when there was severe economic depression and great political unrest in the country. After working for some months as a research fellow at the University of Madras, he joined as a Research Officer in the Agricultural Research Institute,

Coimbatore. It was here that he started his researches on the chemistry of Indian plants.

In 1933, Dr. Seshadri joined the young Andhra University, Waltair, as a Lecturer and Head of the newly started Department of Chemistry. Next year he was appointed as Reader and in 1938 as a Professor. The University entrusted him also with the task of building up and organising the Departments of Chemical Technology and Pharmaceutical Chemistry. Starting from scratch, he laid the foundations for the subsequent growth of these departments to their present stature as amongst the foremost in India. While attending to his responsibilities of teaching and organising, he also found time to pursue research work in the laboratories of the Biochemistry Department of the Andhra Medical College at Visakhapatnam three miles away. His devotion to work inspired many young students to join him for research and make it their life long profession. The Andhra University soon became the most active centre for original chemical research in the country to eventually attain international levels.

The aerial bombardment of the coastal town of Visakhapatnam-Waltair in 1942 during the most intensive phase of World War II necessitated the shifting of the Andhra University College to safer in-land locations. The Chemistry Department functioned for one year at Guntur and for three years at Madras as guest in local institutions. Even under these adverse circumstances research continued and a number of research workers got their research degrees. After the war ended, the Chemistry Department returned to Waltair in 1946 and the rebuilding of the laboratories had to be done all over again since they had been converted into base hospitals for military use.

In 1949 Professor Seshadri was invited by Sir Maurice Gwyer, the then Vice-Chancellor of the Delhi University, to join that University as Professor and Head of the Chemistry Department. His advent into the Delhi University was an important land mark in the history of the Chemistry Department and resulted in enormous expansion of research activities in subsequent years. The department came to be known internationally for its contributions in the area of Chemistry of Natural Products

and in 1963 the University Grants Commission recognised it as a Centre for the Advanced Study of the Chemistry of Natural Products, with Professor Seshadri as its Director. On attaining the age of superannuation in 1965 he was appointed as the first Emeritus Professor of the University.

The decade (1965–1975) following his formal retirement from administrative responsibilities found Professor Seshadri devoting more time and energy than even before for the pursuit of research and he continued to guide young research workers to the day he was removed to the hospital in a critical condition where ten days later, on 27th September, 1975, he breathed his last, following an operation for gastric ulcer.

During the 40-year period of his association with the Andhra and Delhi Universities Professor Seshadri trained over 160 young men and women for doctoral degrees and published over 1100 papers in various scientific journals. A good number of his former students occupy senior positions both in India and abroad in teaching and research establishments. He is the author of a book entitled *Chemistry of Vitamins and Hormones*.

Professor Seshadri's expert advice and mature wisdom were frequently sought by a large number of organisations. He was Chairman/member of various expert committees of the ministries of the Government of India dealing with education, science, health, agriculture and defence, science councils like the Council of Scientific and Industrial Research, Indian Council of Medical Research, Indian Council of Agricultural Research, the Department of Atomic Energy, the Defence Department, the University Grants Commission, and educational institutions and learned societies all over the country. He was also a member of the scientific advisory committee to the Cabinet. He was for some years a Consultant to the UNESCO.

Professor Seshadri was the recipient of numerous honours and awards. He was elected as a Fellow of the Royal Society (London) in 1961. He was conferred honorary doctorate degrees by the Universities of Andhra, Osmania, Venkateswara and Banaras. He was honorary member of the Indian

Science Congress Association. He received the Acharya P. C. Ray Medal and Acharya, J. C. Ghosh medal of Indian Chemical Society, and the Bhatnagar medal and the Meghnad Saha medal of the Indian National Science Academy (formerly called National Institute of Sciences of India). He was at different times President of the Indian Science Congress, of the Indian National Science Academy, of the Indian Chemical Society, of the Oil Technologists' Association of India, of the Indian Pharmaceutical Association and of the Indian Pharmaceutical Congress, Vice-President of the Indian Academy of Sciences, and Chairman of the North India Branch of the Royal Institute of Chemistry. He was an honorary fellow of the Deutsche Akademie für Naturforscher Leopoldina (G.D.R.) and advisor to the London Chemical Society in India. The Government of India conferred on him the Padma Bhushan in 1963.

Professor Seshadri came to his eminent position in the country by virtue of his deep devotion to duty, and sense of dedication to whatever he undertook to do. He was very generous and benevolent to all students and younger scientist colleagues, and fearless in his views on matters concerning science and education and the country's life in general, and most fair to persons who held views different from his own. In spite of his being a scientist of international stature, he was deeply spiritual and was one of the stoutest protagonists of the view that scientific advancement without a spiritual base is not good for individuals or communities including nations. He was rated by his compeers as one of the most eminent, most dedicated and most fearless among scientists of the country, and a singular example of simplicity and humility.

Professor Seshadri was a Vice-President of Current Science Association for some years and continued to be a member of the Association till the end. He was most helpful and prompt as a referee of the journal. In his death the Journal and Indian Science have suffered an irreparable loss, the country has lost one of her noblest sons, and the world a very great scientist.

May his Soul rest in peace.

S. RANGASWAMI.

## LETTERS TO THE EDITOR

### HOMOGENEOUS AND HETEROGENEOUS REACTIONS ON THE DISPERSION OF A SOLUTE IN MHD COUETTE FLOW

RECENTLY Murthy and Murthy<sup>1</sup> discussed the influence of an applied magnetic field in the unsteady dispersion of a solute with simultaneous chemical reaction in a conducting liquid flowing in a channel under uniform pressure gradient. We now consider the same problem for the flow of a viscous incompressible conducting liquid of uniform density  $\rho$ , confined between two horizontal non-conducting planes in the presence of a transverse magnetic field, when the upper and the lower planes  $y = \pm d$  are moved horizontally with equal and opposite velocities  $\pm U_0$  in the direction of the flow along the X-axis and there being no pressure gradient in the liquid. Neglecting the induced magnetic field, the velocity profile in non-dimensional form is given by

$$u(\eta) = \frac{\sinh M\eta}{\sinh M}, \quad (1)$$

where

$$M^2 = \frac{d^2 B_0^2 \sigma}{\rho \nu}$$

is the Hartmann number. Noting that the average velocity is zero, we obtain

$$u_x = \bar{u} - u = -\frac{\sinh M\eta}{\sinh M} \quad (2)$$

In case of diffusion with the combined homogeneous and heterogeneous reaction, we substitute  $u_x$  of eq. (2) in their eq. (15) and solving for C subject to the boundary conditions eq. (16) of their paper, we obtain

$$C = \frac{d^2}{DL} \frac{\partial C}{\partial \xi} \frac{1}{(M^2 - \alpha^2) \sinh M} \times \left[ \frac{M \cosh M + \beta \sinh M}{\alpha \cosh \alpha + \beta \sinh \alpha} \sinh \alpha \eta - \sinh M \eta \right]. \quad (3)$$

The volumetric flow rate at which the solute is transported across the section of the channel of unit breadth is given by

$$Q = \int_{-1}^1 C u_x d\eta. \quad (4)$$

Substituting for C from eq. (3) and  $u_x$  from eq. (2) we obtain Q of eq. (4). Comparing this

with Fick's law of diffusion, we find that the solute is dispersed relative to a plane moving with the mean speed of the flow with an effective dispersion coefficient  $D^*$  given by

$$D^* = \frac{d^2 k_1}{D^2} G(\alpha, \beta), \quad (5)$$

where

$$G(\alpha, \beta) = \frac{1}{4\alpha^2 (M^2 - \alpha^2) \sinh^2 M} \left[ 2 - \frac{\sinh 2M}{M} + \frac{2(M \cosh M + \beta \sinh M)}{\alpha \cosh \alpha + \beta \sinh \alpha} \times \left\{ \frac{\sinh(M + \alpha)}{M + \alpha} - \frac{\sinh(M - \alpha)}{M - \alpha} \right\} \right]. \quad (6)$$

When  $\beta = 0$ , corresponding to the case of diffusion with a homogeneous first order reaction only, we obtain,

$$F(\alpha) = \frac{1}{4\alpha^2 (M^2 - \alpha^2) \sinh^2 M} \times \left[ 2 - \frac{\sinh 2M}{M} + \frac{2M \cosh M}{\alpha \cosh \alpha} \times \left\{ \frac{\sinh(M + \alpha)}{M + \alpha} - \frac{\sinh(M - \alpha)}{M - \alpha} \right\} \right]. \quad (7)$$

We have computed  $F(\alpha)$  for different values of the dimensionless reaction rate parameter  $\alpha$  and for various values of the Hartmann number and entered them in Table I.

TABLE I  
Calculated values of  $F(\alpha)$

| $\alpha \backslash M$ | 0.4    | 0.8    | 1.2    | 1.6    | 2.0    |
|-----------------------|--------|--------|--------|--------|--------|
| 0                     | .78266 | .16548 | .05851 | .02560 | .01274 |
| 1                     | .68412 | .14468 | .05117 | .02239 | .01115 |
| 5                     | .16479 | .03508 | .01253 | .00555 | .00281 |

It is concluded that the effective dispersion coefficient decreases with the increase in the reaction rate along with the increase in the Hartmann number.

We have computed  $G(\alpha, \beta)$  for various values of  $\beta$  and M when  $\alpha = 1$  and entered them in Table II.

TABLE II

Calculated values of  $G(\alpha, \beta)$  when  $\alpha = 1$ 

| $\beta$ | 2      | 4      | 6      | 8      | 10     |
|---------|--------|--------|--------|--------|--------|
| 0       | ·04988 | ·03874 | ·03370 | ·03082 | ·02896 |
| ·5      | ·04802 | ·03724 | ·03235 | ·02956 | ·02776 |
| 1·5     | ·03675 | ·02814 | ·02424 | ·02201 | ·02057 |

In this case also the increase in the wall catalytic parameter together with the Hartmann number causes a decrease in the effective Taylor dispersion coefficient.

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May 1, 1975.

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### ON THE MAGNETIC SUSCEPTIBILITY OF $\text{Nd}_2(\text{WO}_4)_3$

MAGNETIC properties of  $4f$  compounds are of considerable interest in recent years. Many of the  $4f$  compounds such as oxides, orthoferrites, chromates and magnetites are well studied. But the magnetic properties of the tungstates of the type  $\text{In}_2(\text{WO}_4)_3$  ( $\text{In}$  stands for the rare-earth) have not been reported. We are studying the electrical and magnetic properties of the rare-earth tungstates<sup>1,2</sup>. This short note reports our study regarding the magnetic susceptibility of  $\text{Nd}_2(\text{WO}_4)_3$  at high temperatures (300 to 900°K).

The neodymium tungstate was prepared using standard methods. Stoichiometric amounts of pre-fired high purity  $\text{Nd}_2\text{O}_3$  ( $\geq 99.5\%$ ) from Fluka AG Switzerland, and analytical grade of  $\text{WO}_3$  from E. Merck dried at 900°K are mixed thoroughly and heated around 1200°K in a closed platinum crucible for 20 to 24 hours. The reaction products are homogenized by ball milling in an agate mill. It is then pelletized and again fired at 1350 to 1400°K for 6 to 10 hours. This yields a good final product. Neodymium tungstate is light pink in colour. The testing of the materials was done by their melting point. Since the crystal structure of this compound is known<sup>3</sup>, it is easy to test the compound by X-ray powder diffraction technique. The purity of the sample was about 99.8%. The magnetic susceptibility of the powdered specimen has been measured using Faraday's method<sup>2,4</sup>.

The variation of magnetic susceptibility ( $\chi^{-1}$ ) with temperature is shown in Fig. 1. The curve is a straight line suggesting Curie-Weiss law behaviour ( $\chi = C/(T - \theta)$ )

and possible magnetic ordering at low temperatures<sup>5</sup>. From the extrapolation of the straight line and the slope, one can obtain the value of paramagnetic Curie temperature  $\theta = -60^\circ$  and Curie-constant  $C = dT/d(1/\chi) = 3.125 \times 10^{-3} \text{ K/gm}$ .

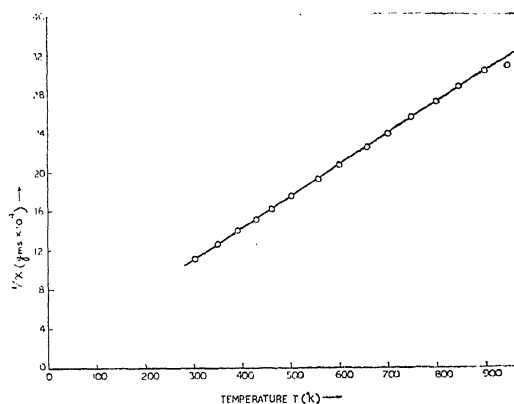


FIG. 1. The inverse of magnetic susceptibility ( $\chi^{-1}$ ) with temperature  $T$  (°K) for the powdered specimen of the  $\text{Nd}_2(\text{WO}_4)_3$ .

The only paramagnetic ion in this compound is  $\text{Nd}^{3+}$ . The ground state of  $\text{Nd}^{3+}$  is  $^4I_{9/2}$ , where  $L = 6$ ,  $S = 3/2$  and  $J = 9/2$  which yields the value of 3.6 for the effective Bohr magneton  $p = g\{J(J+1)\}^{1/2}$  for the ion. From the experimental value of  $C$ , one can calculate the effective Bohr magneton using the standard formula  $p_{\text{eff}}^2 = 3kC/n\beta^2$ , where  $k$  is the Boltzmann constant and  $n$  the number of paramagnetic ions per unit mass. The value obtained for  $p_{\text{eff}} = 3.51$ . There is a good agreement between the theoretical and the experimental values of the  $(p_{\text{eff}})^5$ , indicating the purity of our sample.

The nature of ordering and interaction leading to Curie-Weiss law of high temperature can only be understood by low temperature study of pure and doped samples. However the insulating nature of this compound ( $\sigma \approx 10^{-12} \text{ ohm}^{-1} \text{ cm}^{-1}$  at 300°K) rules out any direct exchange interaction between the magnetic ions. It has also been pointed<sup>7,8</sup> out that other types of exchange interactions in rare-earth ions are very weak and often do not yield ordering at low temperatures. Thus it is quite probable that Curie-Weiss law behaviour, resulting in this compound, is due to crystal field effect with a little contribution from simple dipole-dipole interaction between the magnetic ions. The former may be dominant<sup>9</sup> in view of the less symmetrical structure of the compound.

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Das in preparation of the compound is thankfully acknowledged.

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NASEEB-DAR.  
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### NEW BAND SYSTEM OF CaI MOLECULE IN THE REGION 4600–4950 Å

THE spectrum of CaI molecule has been studied by various workers<sup>1–8</sup> and consists of four band systems, viz., A–X, B–X, C–X and D–X. The ground state of the molecule is a  $^2\Sigma^+$  state. A and C are the  $^2\Pi$  states and D a  $^2\Sigma$  state analogous to the A, C and D stages of the halides of the group II elements. In most of the mono-halides of this group, a few systems in the visible region were observed by various workers and hence, a similar system was expected in CaI also. The spectrum of calcium moniodide molecule in the visible

region was investigated and the results obtained are reported here.

The spectrum of CaI molecule was excited in a high frequency discharge using a 150 W oscillator working in a frequency range of 10–15 MHz. A pure sample of CaI<sub>2</sub> was kept in a conventional type quartz discharge tube. External heating was required to maintain the bright red colour of the discharge. The spectrum was photographed on Ilford R-40 plates in the first order of a two meter plane grating spectrograph at a reciprocal dispersion of about 7.3 Å/mm. An exposure time of about 30 minutes was sufficient to get a good spectrogram. A reproduction of the band system is given in Fig. 1. The measurements of the band heads were made on an Abbe comparator using internal standard.

In the spectrogram a number of violet degraded bands were observed. The most intense group was taken as (0, 0) sequence. Bands corresponding to  $\Delta v = 0, \pm 1, \pm 2$  and  $-3$  were observed with the strong Q and P heads. The bands were analysed as two sub-systems arising from a  $^2\Delta - ^2\Pi$  transition. For the component on longer wavelength side the higher members could not be clearly recognised due to overlapping with the component on shorter wavelength side. The separation between origins of the sub-systems was found to be 57 cm<sup>-1</sup> which is close to the doublet splitting 59 cm<sup>-1</sup> of a state reported by Darji and Vaidya.

The identity of the emitter was established using standard methods. The Q heads of the bands were satisfactorily fitted in the following quantum equations:

$$\nu_{Q_1} = 21670.1 + 287.2(v' + \frac{1}{2}) - 0.80(v' + \frac{1}{2})^2 - 241.7(v'' + \frac{1}{2}) + 0.50(v'' + \frac{1}{2})^2$$

$$\nu_{Q_2} = 21127.1 + 287.2(v' + \frac{1}{2}) - 0.80(v' + \frac{1}{2})^2 - 242.7(v'' + \frac{1}{2}) + 0.50(v'' + \frac{1}{2})^2$$

The analysis reveals that the observed bands belong to two sub-systems of a  $^2\Delta - ^2\Pi$  transition. The lower state vibrational frequency does not agree with

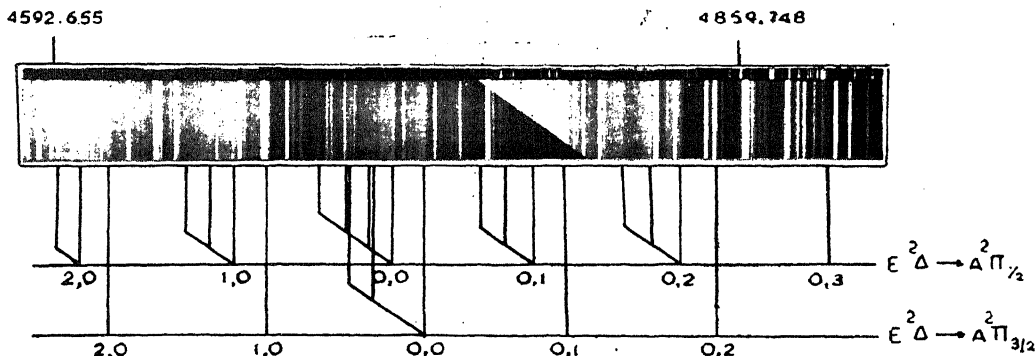


FIG. 1.  $E^2\Delta \rightarrow A^2\Pi$  Band system of CaI molecule recorded at a dispersion of 7.3 Å/mm.

the ground state frequency of CaI molecule ( $238.2 \text{ cm}^{-1}$ ) reported by earlier workers. However, it nicely agrees with the vibrational frequency of the A state ( $241.69 \text{ cm}^{-1}$  and  $242.65 \text{ cm}^{-1}$ ) observed by previous workers. The doublet splitting observed in the present analysis nearly agrees with the reported doublet separation of the A state. Hence it appears that the lower level involved in this transition is not the ground state but the first excited state, viz.,  $A^2\Pi$ . The upper state frequency,  $287.2 \text{ cm}^{-1}$ , is much higher than the frequency of any of the known state of this molecule. In the analogous molecules, it is observed that for a state other than C, the vibrational frequency of the states usually increases as one goes to a higher excited state. The vibrational frequency observed in this case is higher than the frequency of any of the other observed states. The reason may be that the upper state of the system under investigation may be lying well above the previously observed states. If one takes  $A^2\Pi$  state as the lower state of this system, the upper state would lie at about  $36715 \text{ cm}^{-1}$ . The structure of these bands shows that the upper level may be a  $^2\Delta$  state analogous to the  $^2\Delta$  state in CaCl molecule, however, the correct assignment can be done by a rotational analysis only.

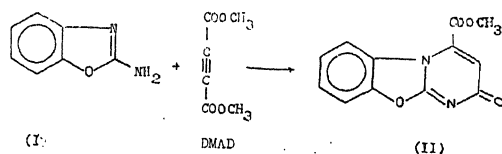
The authors are thankful to Prof. M. M. Patel for suggesting the problem and for useful discussions, Department of Physics, M. N. KAMALASANAN, Faculty of Science, S. G. SHAH, M.S. University of Baroda, Baroda (India), May 5, 1975.

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### REACTION OF DIMETHYLACETYLENE DICARBOXYLATE WITH 2-AMINO BENZO-OXAZOLE

THE reaction of acetylenic esters with heterocyclic compounds have been the subject of several publications in recent years<sup>1,2</sup>. Dimethylacetylene dicarboxylate (DMAD), one of the most versatile acetylenes, has played an important role in organic chemistry, because it undergoes a wide variety of thermal cycle addition and conjugate addition reactions<sup>2</sup>.

The present communication reports the addition of dimethylacetylene dicarboxylate (DMAD) to 2-amino benzo-oxazole (I) yield the cyclised product (II) with elimination of one molecule of methanol. The structure of the product was established on the basis of various spectral and analytical data.



A solution of DMAD (0.01 mole) in methanol was added to a stirred solution of I (0.01 mole) in methanol over a period of 15 minutes in ice-salt bath. The reaction mixture was stirred further for 2 hours at this temperature. The ice bath was removed and stirring was continued overnight at room temperature. The resulting product was filtered, washed with methanol and recrystallised from methanol. It gave a light yellow crystalline solid (II) (1.71 gm, 70%) m.p.  $180^\circ$ .

Found: C, 58.74, H, 3.8, N, 11.7.  $C_{12}H_8N_2O_4$  requires C, 59.01, H, 3.27, N, 11.4.

Molecular weight = 244. U.V. spectrum (Ethanol, nm): 315, 242. I.R. (Nujol,  $\text{cm}^{-1}$ ) 1740 s (ester), 1635 s, 1600 s, 1270 s (C-N), 1175 s.

NMR (60 Hz,  $\text{CDCl}_3$ ; TMS;  $\delta$  values): At 7.5 to 8.3 (4 H, broad multiplet; aromatic protons); one sharp singlet at 7.15 (1 H, olefinic proton); one sharp singlet at 4.25 corresponding to carbo-methoxy group (3 H,  $-\text{OCH}_3$ ).

The authors are thankful to Dr. H. S. Sachdev, Harvard University, U.S.A., for guidance and to Prof. G. B. Singh and Prof. O. P. Malhotra, for providing necessary facilities and to the C.S.I.R., New Delhi, for financial help.

Dept. of Chemistry, MAHENDRA NARAIN SHARMA,  
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## STIMULATION OF GAS PRODUCTION FROM COWDUNG BY ALGAL SUPPLEMENTATION

THE production of combustible gas, methane, by cowdung is based on its anaerobic fermentation in a closed system. The availability of raw material, simplicity of design and operation, adaptability in rural areas and the manurial value of the residual slurry are the chief economic advantages of the bio-gas plants<sup>1</sup>, particularly during the present shortage of natural gas. With a daily charging schedule, gas production in these plants varies from 1.4 cft/lb cowdung in the summer months (*ca* 30° C) to about 0.6 cft/lb in winter (*ca* 15° C)<sup>1,2</sup>. Augmentation of gas production in the existing prototypes by inexpensive additives will certainly be advantageous in improving the efficiency of these systems. This preliminary report deals with the stimulation of gas production by cowdung by algal supplementation.

The sun-dried algal mixture used in the present experiments was harvested from the Pirana sewage oxidation ponds at Ahmedabad. The predominant algal member in this mixture was *Oscillatoria chalybea*, which formed a bloom in these ponds. The other algae were species of *Euglena*, *Scenedesmus*, *Spirulina* and *Merismopedia*, which were all sparse in distribution.

Laboratory fermentation of cowdung was carried out in a battery of 10 litre aspirator bottles, each containing 4 kg fresh cowdung made into a slurry with 4 litre of water. The gas was collected over saturated brine solution and measured by liquid displacement. The ambient temperatures at which the experiments were conducted were 30–31° C, 20–22° C and 16–18° C. In one series, the cowdung was supplemented with 3% dried algae.

The time-course curve for gas production by cowdung alone at 30–31° C showed a steady increase (Fig. 1, C), followed by a decline (Fig. 1 B, C). Incorporation of 3% dried algae into the dung stimulated gas production considerably (Fig. 1, CA). At the end of 10 days, the total gas produced in the algal supplemented series was about 1.64 times more (3114 cm<sup>3</sup>/kg dung) than in dung alone (1900 cm<sup>3</sup>/kg). Between the fifth and sixth day, the unit amount of gas produced per day was almost three times in the algalized series (Fig. 1 B, CA).

When the temperature dropped to 20–22° C, the gas production by the cowdung was only about half (1080 cm<sup>3</sup>/kg) of that obtained at 30–31° C and with a further fall in the temperature (16–18°), the reduction in gas production was almost four fold (432 cm<sup>3</sup>/kg) (Fig. 1).

At 20–22° C, algal supplementation resulted in about 60% increase in the total gas produced at the end of the experimental period over the unsupplemented series (Fig. 1). At 16–18° C, the rate of gas production in the algalized series was equal to that at 20–22° C without algal addition (Fig. 1). Thus, in terms of gas production, addition of algae to cowdung was found to compensate the effect of 4° C difference at low temperature ranges.

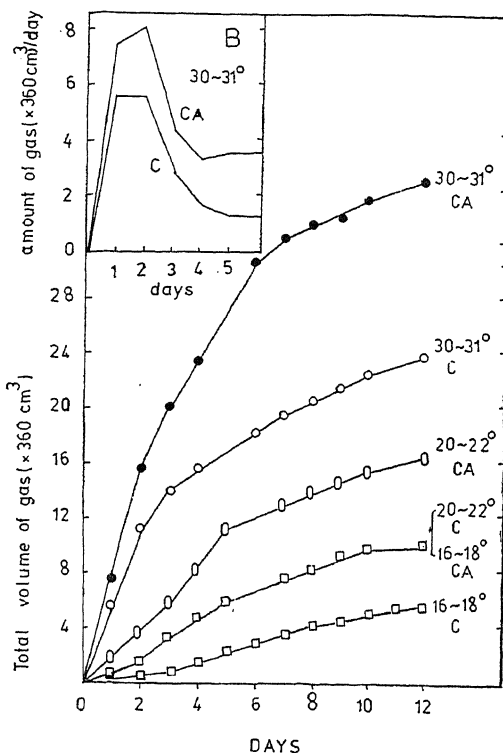


FIG. 1. Gas production by cowdung with (CA) and without (C) algal supplementation at different ambient temperatures; B, rate of gas production per day by cowdung with (CA) and without (C) algae at 30–31° C.

There was no appreciable difference in the carbon dioxide concentration of the gas produced by the dung in the presence (35–40%) and absence (30–38%) of algae. Nor there was any difference in the combustibility.

Algae will thus be useful as an inexpensive additive to increase the efficiency of the existing prototypes of the cowdung gas plants, for they are required in small amounts and can be grown in sewage oxidation ponds.

We are grateful to Dr. A. B. Joshi for his keen interest and suggestions; to Dr. N. S. Subba Rao for



facilities and to Mr. M. V. Srinivasan for supplying the sewage grown algal material.

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June 13, 1975.

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### MICROPLANKTONS FROM TETHYAN SEQUENCE OF NITI PASS, KUMAON HIMALAYAS, INDIA

DURING the course of microfaunal (microfloral) study of a number of Paleozoic and Triassic samples of the Paikhandia region, the rock analyses revealed a fair occurrence of microplanktons (acritarchs and tasmanitids) in association with sporadic distribution of disaccate pollen grains.

The present paper reports the occurrence of microplanktons (acritarchs and tasmanitids) from the Silurian, Carboniferous, Permo-Carboniferous and Triassic sequences exposed at southwest and near the Niti Pass in Paikhandia, Kumaon Himalayas, India. Their presence around the Niti Pass is of considerable interest with reference to the paleo-environmental interpretation of the sequences. In addition, it is also suggestive of further detailed work to be carried out on the Paleozoic and Triassic sequences for biostratigraphic zonation and paleoecological study.

The material investigated was collected by B. S. Jangpangi and D. P. Dhoundal in the summer of 1953 for the University of Lucknow, Lucknow.

#### Description

#### 'INCERTAE SEDIS'

Group .. Acritarcha Evitt, 1963.

Sub-group .. Sphaeromorphitae Downie Evitt, and Sarjeant, 1963.

Genus .. *Leiosphaeridia* (Eis.) Downie and Sarjeant, 1963.

*Leiosphaeridia* cf. *L. microgranifera* (Staplin) Downie and Sarjeant, 1963 : Test circular; thin test wall provided with microgranules (diameter about  $0.5 \mu$ ); test diameter about  $45 \mu$ .

*Leiosphaeridia minuta* (Staplin) Downie and Sarjeant, 1963 : Test subcircular; test wall smooth without folds; thin-walled; test diameter range 18 to  $20 \mu$ .

*Leiosphaeridia orbiculata* (Staplin) Downie and Sarjeant, 1963 : Test circular; test wall smooth with 2 to 3 folds, relatively thick-walled (thick-

ness range 2 to  $3 \mu$ ); test diameter range 24 to  $51 \mu$ .

*Leiosphaeridia* cf. *L. wenlockia* Downie, 1959 : Test subcircular; test wall smooth with a few folds; wall thickness range 1 to  $1.50 \mu$ ; test diameter range 24 to  $45 \mu$ .

*Leiosphaeridia* sp. 1 : Test sub-spherical; test wall smooth with 2 to 3 folds present, wall thickness about  $1 \mu$ ; test diameter about  $60 \mu$ .

Sub-group .. Netromorphitae Downie, Evitt, and Sarjeant, 1963.

Genus .. *Quisquilites* Wilson and Urban, 1963.

*Quisquilites buckhornensis* Wilson and Urban, 1963 : Test bilaterally symmetrical, bean-shaped; test wall translucent to transparent, three-layered, outside layer smooth or with minute pores, walls show various stages of deformation. Dimension of a specimen : length  $102 \mu$ , width  $78 \mu$ , wall thickness  $3 \mu$ .

Sub-group .. Tasmanitidae (Sommer) Staplin *et al.*, 1965.

Genus .. *Tasmanites* (Newt.) Eisenack, 1958.

*Tasmanites* sp. 1 : Test circular; test wall with micropores (diameter range 2 to  $3 \mu$ ), wall thickness range 5 to  $6 \mu$ ; test diameter range 110 to  $150 \mu$ .

*Tasmanites* sp. 2 : Test circular; test wall with micropores (about  $1.50 \mu$  in diameter); wall rupture producing a cryptosuture; test diameter  $79 \mu$ .

#### Distribution

The Kumaon collection belongs to six different localities from the Silurian, ranging from Gh. 5 (Lower Silurian) to Gh. 9 (Upper Silurian shales with brachiopods). The microplankton-bearing horizon is Gh. 6 of limestones with brachiopods, yielding microplankton *Leiosphaeridia orbiculata* (Fig. 1) and the sample was collected from the Pa'apani locality. Shale sample with brachiopods (Gh. 9 horizon) of Hrudhar locality yielded *Leiosphaeridia minuta* and *L. orbiculata*.

The Kumaon rocks of eight different localities from the Carboniferous ranges from Gh. 12 (Lower Carboniferous) to Gh. 19 (Upper Carboniferous quartzites). The microplankton-bearing horizon is Gh. 13 of bryozoa limestone of the Gerdung and Kaljabar (Paikhandia) region and yielded *Leiosphaeridia minuta*, *L. orbiculata*, *L. cf. microgranifera*, and *Leiosphaeridia* sp. 1 (Fig. 1).

The rocks of the eight different localities from the Permo-Carboniferous of the Kumaon region range from Gh. 20 (Lower Permo-Carboniferous) to Gh. 27 (Upper Permo-Carboniferous sandstone with brachiopods). The microplankton-bearing horizon is Gh. 20 of coral limestone in the

Kuinglang (Painkhanda) region and yielded *Leiosphaeridia minuta*, L. cf. *microgranifera*, and *L. orbiculata*. The shales with brachiopods (horizon Gh. 23) of the Kaljabar (Painkhanda) region revealed the microplanktons *Quisquilites buckhornensis* and *Tasmanites* sp. 1.

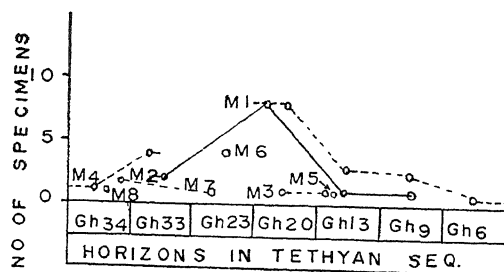


FIG. 1. Quantitative composition of organic-walled microplanktons (acritarch and tasmanitid) in rocks representing each horizon in Tethyan sequence (Gh. 6 to Gh. 34; Lw. Silurian to Up. Triassic) of Niti Pass, Kumaon Himalayas. (M 1, *Leiosphaeridia orbiculata*; M 2, *L. minuta*; M 3, *L. cf. microgranifera*; M 4, *L. cf. wenlockia*; M 5, *Leiosphaeridia* sp. 1; M 6, *Quisquilites buckhornensis*; M 7, *Tasmanites* sp. 1; M 8, *Tasmanites* sp. 2.

The rocks from seven different localities from the Triassic of Niti Pass range from Gh. 28 (Lower Triassic) to Gh. 34 (uppermost Triassic, i.e., the Lower Megalodon Limestone). The microplankton-bearing horizon is Gh. 33 of limestones with Belemnites of the Barahati region and consists of *Leiosphaeridia minuta* and *L. cf. wenlockia*. The fossiliferous limestones and shales (horizon Gh. 34) of the Chhotahots region revealed *Leiosphaeridia* cf. *wenlockia* and *Tasmanites* sp. 2.

Author is thankful to his colleague Dr. A. Sahni for suggesting the investigation of rocks and to Prof. S. N. Singh (Department of Geology, University of Lucknow) for providing facilities.

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# HISTOLOGY OF THE ADRENAL GLAND OF THE INDIAN FALSE VAMPIRE BAT, *MEGADERMA LYRA LYRA* (GEOFFROY)

MAMMALIAN adrenal gland is unique among vertebrates in that the steroidogenic and chromaffin cells are clearly separated as cortex and medulla respectively. Further, the cortex is clearly demarcated into three histologically distinct zones namely, zona glomerulosa, zona fasciculata and zona reticularis, each with a distinct pattern of cellular arrangement and function<sup>1-3</sup>. However, the same is said to be

not true about chiropteran adrenals. The available reports indicate that there is no cortical zonation in most of the species studied<sup>2-4</sup>. There are no reports on adrenal histology of the Indian chiropterans and so the present investigation was undertaken.



FIGS. 1-2. Fig. 1. T.S. of adrenal, outer region to show the cellular details of zona glomerulosa and zona fasciculata. Arrow indicates penetration of glomerular cells into fasciculata. 10 × 40. Haematoxylin-eosin. Fig. 2. T.S. of adrenal, inner region to show the cellular details of zona reticularis and medulla. 10 × 40. Haematoxylin-eosin. (c = capsule; g = glomerulosa; f = fasciculata; m = medulla.)

Adrenals from *Megaderma lyra lyra* collected at Srirangapatna (India) were fixed in Bouin Hollande sublimate and paraffin sections cut at 8 μ thick were stained in haematoxylin-eosin and Mallory's triple for histological observations.

The adrenals occupy an anteromesial position and are closely applied to the kidney. The shape

varies from pyramidal form to cylindrical form. Lobulations and infoldings are not observed. The gland is surrounded by a connective tissue capsule which consists of spindle-shaped fibrous cells (Fig. 1). Connective tissue strands extend from the capsule into the cortex. There is a clear separation of medulla from the cortex. The three zones of the cortex are clearly distinguishable (Figs. 1 and 2). Zona glomerulosa consists of comparatively small spherical cells which are compactly arranged and the cytoplasm stains lightly. The glomerular zone varies in thickness and the cells penetrate into the fascicular zone at certain points (arrow in Fig. 1). Zona fasciculata consists of large irregularly polyhedral cells arranged in radiating strands. The cells are relatively larger and the cytoplasm stains darkly. The transition between fasciculata and reticularis is gradual. In zona reticularis the cells are disposed in the form of anastomosing cords. Medulla is a distinct unit. The medullary cells are arranged in irregular strands and masses interspersed by blood vessels and sinusoids. A connective tissue envelope surrounds the individual strands and masses of medullary cells.

Rudd, cited by Gorbman and Bern<sup>2</sup>, observed that there was no clear zonation in the adrenal cortex of *Eptesicus fuscus*. But medulla was a distinct unit in this species. Contrary to this a clear separation of medulla from the cortex was not found in the adrenals of *Antrozous pallidus*, but a distinct zona reticularis was noticed in the same species<sup>2</sup>. Bourne<sup>4</sup> after examining the adrenals of 25 species pointed out that it is very difficult to recognise any zonation in most of the species he studied. But in the present investigation a clear zonation in the adrenal cortex of *M. lyra lyra* can be clearly seen.

We are indebted to Prof. M. R. Rajasekarasetty for his encouragement and to C.S.I.R. (India) for awarding a Research Fellowship to one of us (B. S. B.).

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## THE STRUCTURE AND FUNCTIONS OF THE TRUNCUS ARTERIOSUS IN *RANA TIGRINA* (DAUD)

THOUGH frog is the main vertebrate of study in biology, physiology and pharmacology, the structure and function, and particularly the flow of blood in the heart, are inadequately described in spite of several investigations<sup>1-5</sup>. The present study describes the structure and functions of the truncus arteriosus of the Indian frog *Rana tigrina* with particular reference to flow of blood.

### Material and Method

Frogs collected from the vicinity of Lucknow were narcotized by the administration of 5 ml of 10% Urethan solution through the dorsal lymph sinus. The heart was exposed and 0.046 ml of 1% Fluorescein-Ringer's solution was slowly injected into the right auricle using Alga micrometer syringe. The course of blood in the truncus and the aortic arches was followed as per description by Graaf<sup>6</sup>. Ultra-violet illumination was used for observing the course of blood through the truncus arteriosus and the aortic arches. Preserved frogs were used to study the gross anatomy of the heart.

### Present Findings

The conus arteriosus of *Rana tigrina* arises from the right dorsal side of the ventricle (Figs. 1 and 2).

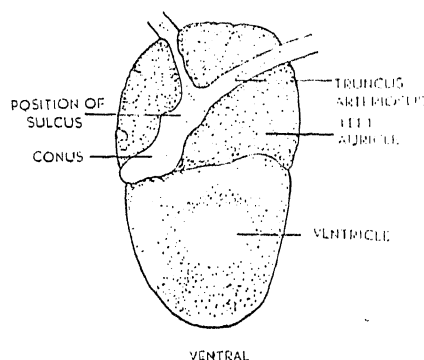


FIG. 1. Diagram of the heart of *Rana tigrina*, ventral view,  $\times 2$ .

It was well demarcated from the truncus arteriosus by a sulcus. Whereas the conus is muscular and thicker than the truncus, the latter has no cardiac muscles and is lower in diameter. The truncus bifurcates into two trunks, the right trunk is more dorsally placed and on the ventral side of the heart. Opening the conus and truncus arteriosus by midventral incisions, the hammer-shaped spiral valve in the conus and the dorsally placed opening of the pulmocutaneous arches, slightly to the left of the middle line and below (in fact dorsal) the head of the spiral valve, are

exposed. A flattened partition divides the recess or lumen of the truncus arteriosus. Sharma<sup>3</sup> described this partition as a 'pad-like valve'. This partition hereafter called *septum principale* is attached to the dorsal wall of the recess of the truncus (Fig. 3).

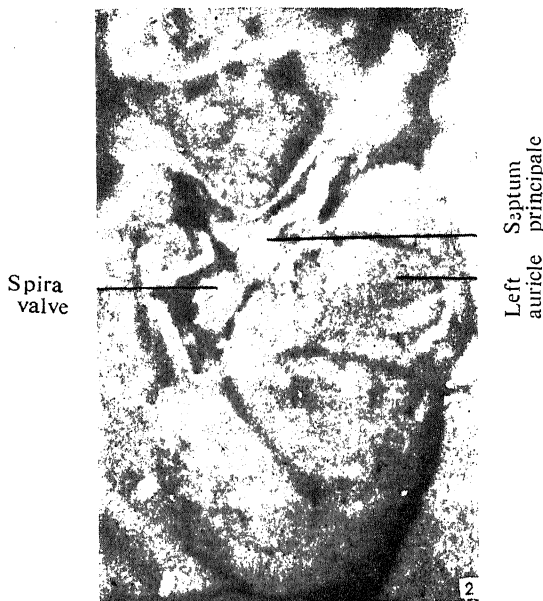


FIG. 2. Photograph of the heart of *Rana tigrina*, conus and truncus are opened to show the spiral valve, the septum principale, the septum mediale and the openings leading into the arterial arches,  $\times 4$ .

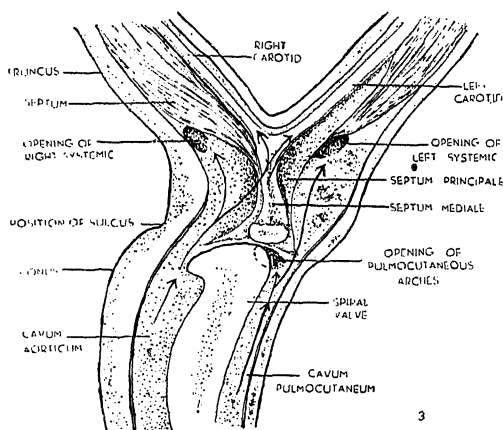


FIG. 3. Diagram of the conus and truncus of *Rana tigrina* showing the course of blood into the arches, via conus and truncus,  $\times 10$ .

A median ridge, the *septum mediale*, runs cranio-caudally and obliquely from the right to the left of the ventral side of the septum principale. The ridge terminates into a flattened knob that lies freely

in the lumen of the truncus, very close to the right half of the spiral valve. On the right side of the septum mediale is a longitudinal fold-like valve guiding distally into the carotid arches of both sides. Entry to the carotid arches of both sides, till now, was considered separate from the other.

Carotid arches of both sides are separated from the systemics by large fleshy longitudinal partitions. The opening of the right systemic lies on the far right side of the septum principale, and that of the left systemic on the left of the septum principale and close to it.

#### Discussion

For long it was believed that the blood in the ventricle of frog is prevented from mixing by means of trabeculae of ventricular wall and when the ventricular contraction begins, the first blood to leave it is the deoxygenated blood having come from the right auricle. This blood soon finds its way into the pulmocutaneous arches and is sent to lungs and skin for oxygenation. The spiral valve obviously prevents the remaining blood of the ventricle from entering the pulmocutaneous arches. Soon after, the spiral valve changes its position to allow the blood from the middle of the ventricle to pass mainly in the systemic arches. The blood of the ventricle yet does not go to the carotids because the carotid labyrinths resist the blood-flow into these arches; only when the pressure finally rises with the ultimate contraction of the ventricle, does the blood flow into the carotids. The blood was well oxygenated as it was received from the left auricle.

Vandervael *et al.*<sup>1</sup> however demonstrated that there is no separation of oxygenated and deoxygenated bloods in the frog's heart and that all the three arches receive a mixture simultaneously. They attached no importance to the spiral valve so far as the blood-flow was concerned.

Simons<sup>2</sup> found in a number of species of frogs and toads (but not in *Rana tigrina*) that the blood from the left of the ventricle passes up one side of the spiral valve of the conus into the carotid arteries and the right systemic, while the blood from the right side of the ventricle passes mainly up the other side of the spiral valve into the pulmocutaneous arteries and also in the left systemic arch. During the earlier part of the systole, a stream of blood flows over the spiral valve from the cavum aorticum into the cavum pulmocutaneum and then on to the left systemic, thus ensuring that some 'arterial blood' does find its way into the left systemic arch.

Working on *Rana tigrina* Sharma<sup>3</sup> believed that the blood from the right side of the ventricle (least

oxygenated) is sent to pulmocutaneous arches, and the blood from the left side of the ventricle (most oxygenated) is sent to the carotids and the systemics. He envisaged no difference between carotids and systemics. Sharma<sup>3</sup> made a mention of the 'pad-like valve' in the truncus of *Rana tigrina* and gave no importance to it in the flow of the blood. The author of the present note has found that this structure is a definite partition in the truncus. The median ridge, i.e., the septum mediale, fairly reaches the spiral valve and there is every possibility that, during the contraction of the ventricle and the conus, the septum mediale forms an effective partition in the truncus, thus cutting off the blood passing on the two sides of the spiral valve. Consequent upon this effective partition, the blood from the left side of the ventricle passes up into the right and left carotids and also into the right systemic, for, their openings lie on the right side of the septum mediale; and the blood from the right side of the ventricle passes up mainly into the pulmocutaneous arches and also into the left systemic arch whose opening lies on the left side of the septum mediale (Fig. 3). This finding by the present author has been confirmed from the observations made under ultraviolet illuminations and is quite contrary to the views of Sharma<sup>3</sup> and identical to those of Simons<sup>2</sup>.

The author is grateful to the management of Lucknow Christian College for providing many facilities for the work. The author also thanks S. C. Shrivastava, D.Sc., of Lucknow University, for his active collaboration and guidance, and also Dr. W. E. Bauer for the photographic work in this college.

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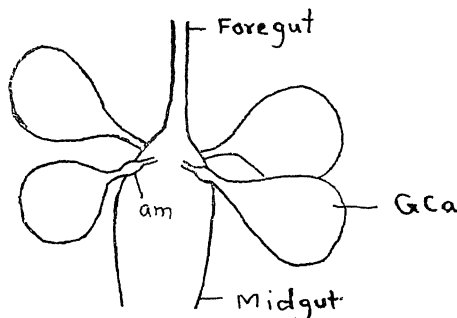
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#### FIRST RECORD OF CAECAL DIVERTICULA IN *SAGRA FEMORATA* DRURY. (SAGRINAE: COLEOPTERA)

*Sagra femorata* Drury occupies a unique position amongst the destructive insect pests of the common bean, *Dolichos lablab* at Jabalpur. The pest was first recorded in Jabalpur during 1969 by Rawat and Jakhmola (unpublished) who have found it as

a pest of regular occurrence on common bean. The present study was undertaken at the College of Agriculture, Jabalpur, during 1973-74.

The freshly killed full-grown larvae of *S. femorata* were dissected in water under binocular for the study of caecal diverticula. A remarkable development of diverticula on the anterior portion of ventriculus occurs in the larva. There are four



glandular, bladder like gastric caecae (GCa), connected circularly around the ventriculus by a thin duct which has got small ampula (am) at its base. They are situated laterally in mesothorax, a pair on either side and richly supplied by the tracheae from the first abdominal spiracle. Kulkarni (1973) observed four white spherical gastric caecae which arise at the union of foregut and midgut of *Scelodonta strigicollis* (Motschulsky), a chrysomelid. Snodgrass (1935) mentioned that blind pouches varying in number and in length may be developed on different parts of the ventriculus. Most commonly they occur at the anterior and surrounding the stomodaeal valve. The present study is the first record in the sub-family Segrinae.

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# CYTOLOGICAL EFFECTS OF FUNGICIDES, PLANTVAX AND VITAVAX ON SOMATIC CELLS OF *ALLIUM CEPA*

INCREASED utilization of fungicides for crop protection in modern agriculture has raised the question whether these chemicals induce detectable cytological damage to the cells. Growing awareness of the importance of these effects has led to several investigations regarding the chromosomal aberrations and mutations resulting from the application of insecticides, fungicides and herbicides<sup>3-5</sup>.

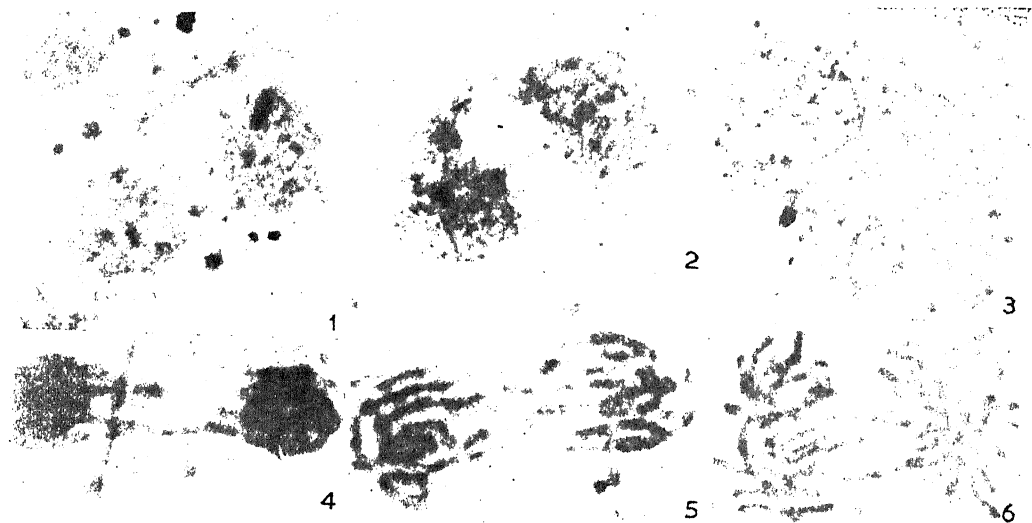
In the present study vitavax (5,6-dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide) and plantvax (5,6-dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide-4,4-dioxide) two systemic fungicides are tested for their cytological effects on onion root tip cells.

Onion bulbs with intact roots were treated with 0.5% solutions of these chemicals for 1 hr at 26° C and the roots were fixed in 1 : 3 acetic alcohol after washing thoroughly in water. Roots of a bulb immersed in water for the same period served as control. Squashes were made of Feulgen stained root tips and observations were recorded on 500 cells from three different root tips.

TABLE I  
Mitotic index and frequency of cytological  
aberrations (%)

| Treatment | Mitotic index | Binucleate cells | Anaphase cells with bridges | Telophase cells with bridges | Polyploid cells |
|-----------|---------------|------------------|-----------------------------|------------------------------|-----------------|
| Control   | 25.0          | ..               | ..                          | ..                           | ..              |
| Plantvax  | 6.0           | 5.0              | 30.0                        | 10.0                         | 4.0             |
| Vitavax   | 6.8           | ..               | 15.0                        | 2.0                          | ..              |

(Fig. 2-6) and pycnotic cells observed in both the treatments. Plantvax treatment has resulted in the induction of polyploidy (Fig. 3) and binucleate cells (Fig. 1). Differential contraction of chromosomes was observed in both the treatments. In general, the cytological effects of these fungicides resemble the aberrations caused by radiation and other mutagenic treatments. Radiomimetic properties similar to those observed with these fungicides were reported with some other pesticides also<sup>1,2,5</sup>.



FIGS. 1-6. Mitotic stages (1-3 : Plantvax treated ; 4-6 : Vitavax treated). Fig. 1. Binucleate cell. Fig. 2. Early telophase with chromosome bridges. Fig. 3. Polyploid cell. Fig. 4. Telophase bridge. Figs. 5, 6. Anaphase bridges.

The data presented in Table I show that the mitotic index is significantly lower in the treated cell population compared to the control and there are significant differences in mitotic index between the two fungicidal treatments. However, plantvax treatment has resulted in greater frequency of abnormal anaphase and telophase cells compared to that of vitavax. Besides, the chromosome bridges fragments

Persistence and frequency of these aberrations in meiotic cells and the effect of different modes of fungicidal application are under study. Information on the cytological damage and recovery due to the widely used fungicides, pesticides and herbicides on different crop plants will be useful not only from the viewpoint of understanding the mechanisms of cytological damage and recovery but

also for its implications in environmental pollution.

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#### EFFECT OF HYDROGEN-ION CONCENTRATION ON THE ACTIVITY OF INCLUSION BODIES OF *SPODOPTERA LITURA* NUCLEAR POLYHEDROSIS VIRUS

THE subtle influence of pH on the activity and dissolution of polyhedral inclusion bodies (PIB) of nuclear polyhedrosis virus (NPV) of *Heliothis zea* and *Trichoplusia ni* has been reported earlier<sup>1,2</sup>. The present communication deals with the effect of different levels of pH on the activity of PIB of *Spodoptera litura* F.

The purified suspension of PIB of *S. litura* NPV obtained by differential centrifugation was brought to different pH levels<sup>3</sup>, viz., 9.8, 8.4, 7.0, 5.6 and 3.8 by mixing with appropriate buffers. Virus with a concentration of  $4.8 \times 10^6$  PIB/ml in the respective buffers was incubated at 28° C for one day and fed to twenty early fourth instar larvae along with host leaf. Larvae fed with virus-free leaf dipped in phosphate buffer, pH 7.0, served as control and mortality counts were recorded at regular intervals.

The study revealed that at neutral pH (7.0), highest larval mortality (100%) was recorded within 4.5 days. At pH 8.4 and 5.6, despite a general decrease in the larval mortality (60 and 50%); the activity remained unaffected. Three to four fold reduction in the activity of the virus was obtained at pH 9.8 and 3.8 which concomitantly prolonged the mortality time, through 8.7 to 9.0 days with 30 and 20% mortality respectively. Admittedly, cent per cent pupation was recorded in control.

The present results add additional support to the views of Bergold<sup>4</sup> who stated that the pH of the suspending media should not drop below 5.0 or rise above 8.5 in order to prevent the dissolution of PIB of NPV of different insects. Falcon<sup>5</sup> suggested that basic pH of the cotton leaf surface

might inactivate the NPV of *Heliothis* virus; in the present study it was observed that even though the tissue pH of castor leaf was 5.9, it did not affect the activity of the virus when the latter was brought to pH 7.0. It evidently shows that the tissue pH does not materially alter the activity of the virus. The results presented here clearly indicate that the virus should be maintained at near neutral pH for the effective control of insects.

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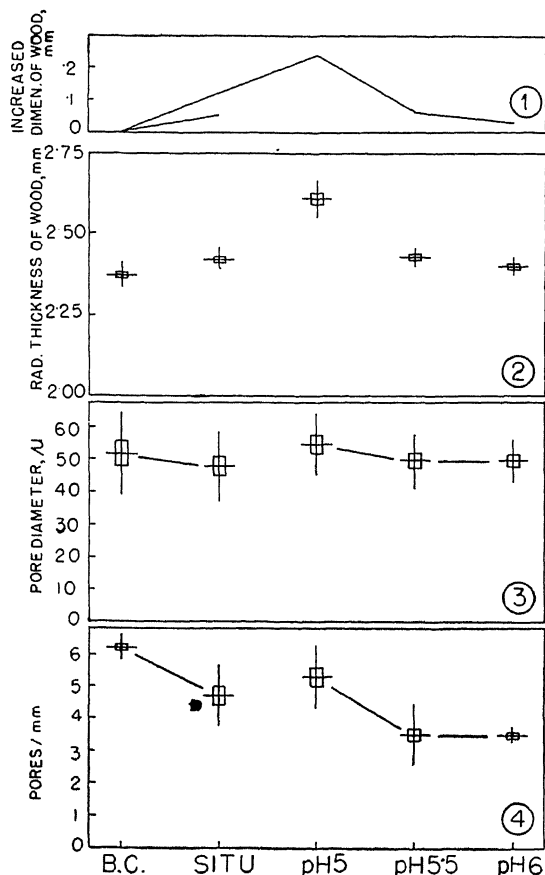
#### IN VITRO EFFECT OF ACIDITY LEVELS ON XYLEM DIFFERENTIATION IN PLUMERIA

DIFFERENTIATION in secondary xylem elements from cambium is a complex problem. The factors controlling differentiation of so many different types of cells from only two types of genetically identical initials are puzzling to morphogeneticists. Culture methods were applied for determining factors that induce xylem formation and phloem formation<sup>4,5,10,11</sup>. Earlier different hormones were tried on *Plumeria* and *Adhatoda* wood<sup>6,8</sup>, in order to know why different types of cells are produced. The present study relates to the effect of pH levels on the differentiation of cell types in the xylem.

Young branches (2.5–3 cm dia.) of the plant (*Plumeria rubra* Linn. var. *acutifolia* Bailey), growing in the University garden, were collected. After removing bark, the wood pieces (8 × 4 mm), covered by the cambium zone were cut from the young branches at a distance of 75 cm from the tip. The pieces were placed aseptically with the cambium attached to the static medium of Schenk and Hildebrandt<sup>7</sup> with 0.1 mg/l of IAA (in place of 2, 4-D) at different pH levels (5, 5.5, 6, 15 in each. Fifteen wood pieces from the same branch levels, as treat ones were preserved in FAA for future study of the preculture condition. Branches were tagged at a distance of 75 cm from the tip in the same growing plant and were allowed to grow *in situ*,

Anatomical characters of the superficial layer of wood were studied after nine weeks. Data were collected from about 15 samples of each of the 5 treatments, 15 samples of preculture wood and 15 wood pieces collected from the tagged portions grown *in situ*.

It was evident that pH 5 caused the highest increase of radial wood thickness while pH 5.5 and 6 increased the xylem almost equal to that grown *in situ* (Figs. 1 and 2).

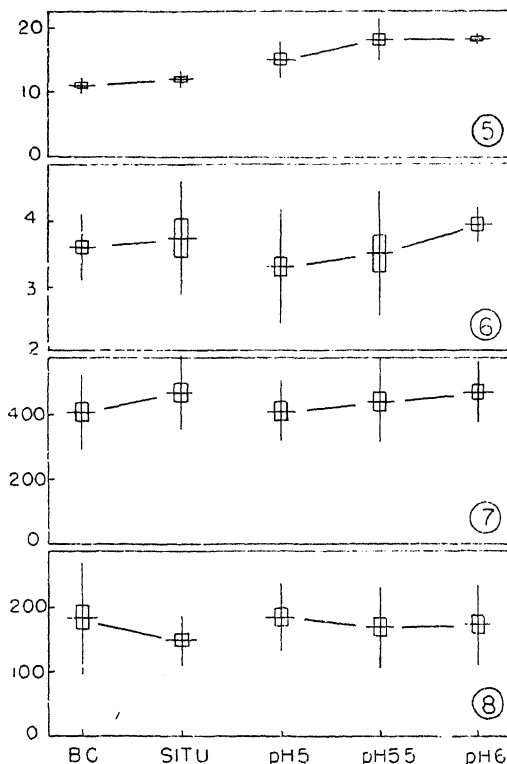


FIGS. 1-4. Measurements of secondary xylem and elements of *Plumeria rubra* var. *acutifolia*. Fig. 1. Increase in the radial dimension of wood after culture and after *in situ* growth; Fig. 2. Measurement of radial thickness of wood; Fig. 3. Tangential diameter of pores; Fig. 4. Number of pores per mm on the circumference of the recent wood.

(B.C. = before culture; SITU = wood grown *in situ*. Mid transverse lines indicate mean values, vertical lines, standard deviations, and upper and lower limits of rectangular boxes, the standard errors.)

Pore diameter (Fig. 3), pore frequency (number of pores per mm on the wood circumference) decreased

with rise of pH as well as after growth *in situ* (Fig. 4). Abundance of parenchyma (Fig. 5) and rays (Fig. 6), and the length of vessel members (Fig. 7) increased with the increase of pH as well as with *in situ* growth. Ray height (Fig. 8) decreased with the rise of pH level and with *in situ* growth. This relation of differentiation in the pH levels of culture media and that of *in situ* condition suggests the possibility of corresponding increase of pH *in situ*.



FIGS. 5-8. Measurements of secondary xylem and elements of *Plumeria rubra* var. *acutifolia*. Fig. 5. Number of parenchyma per mm on the circumference of recent wood; Fig. 6. Number of rays per mm counted similarly; Fig. 7. Vessel member length in μ; height of uniseriate rays in μ. (Indications as in Figs. 1-4.)

Estimation of pH of the aqueous extracts of preculture wood and the wood grown *in situ* for nine weeks showed that the former had 5.5-5.2 pH values and the latter 5.8 to 6.2. Thus it was evident that pH changed in the wood during growth *in situ*. This change had a clear relation to the differentiation of elements. *In vitro* experiments would be a very useful way of studying the acidity-alkalinity factors determining xylem differentiation.



Decrease of the pore diameter and pore frequency was related to increase of the frequency of parenchyma and ray. The number of parenchyma might easily increase by more involvement of fusiform initials in the differentiation of parenchyma cells and less involvement in the formation of vessel members.

The question is, how ray frequency increased. Can this change of pH alter the initials also? According to many authors<sup>1-3,9</sup> a new ray may arise from a fusiform initial by: (1) the cutting off of a cell from the tip which then function as a ray initial; (2) septation of an entire fusiform initial to form a vertical series of ray initials; and (3) division of existing rays by intrusion of elongating fusiform initials.

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### SOMATIC VARIATION IN *CAJANUS CAJAN*

OUTCROSSING in the papilionaceous pigeonpea (*Cajanus cajan* Millsp.) is reported to be less than 10%<sup>1</sup> while the offtypes in true breeding varieties frequently range from 10 to even 30%. So far, there appears to be no explanation for this discrepancy except to conjecture that outcrossing has been of a much higher magnitude than reported. The following observations clarify this discrepancy

and point out to an additional source of variation for improving this important food legume.

### Observations

(1) In a field population of Hy-3 A pigeonpea bred at this Station, multiple 'seedlings' were observed and suspicion arose that they may be polyembryonic in origin. To confirm this, seven varieties of pigeonpea, T-21, Hy-1, Hy-2, Hy-3 A, Hy-3 B, Hy-3 C and Hy-4 were germinated in rectangular trays at room temperature. Upon initiation of germination, a single radicle and then the plumule emerged normally; 6-8 days later, two more side shoots emerged from the cotyledonary axils in most seeds. The phenomenon was common to all varieties and the mean percentage of seeds which produced three shoots per seed ranged from 60-80% in the laboratory. Germination of pigeonpea is hypogeal and under field conditions, the percentage of seeds with more than one shoot ranged between 5 and 15% only, obviously due to environmental restrictions.

(2) When such seeds with multiple shoots were transplanted and raised to maturity, in some of the cases, one of the side shoots from the same seed was observed to be morphologically different from its sister shoots. There was no difference in chromosome number between the sister shoots and meiosis was regular. The differences between sister shoots involved mass changes covering maturity, leaf size, flower, pod and seed size, and plant pigmentation resulting in one of the sister shoots being a total variant.

(3) In a field population of *Cajanus* types, a number of plants exhibited chimeral variation like the occurrence of a determinate branch on an indeterminate type. The determinate sports conformed to the description given by Reddy and Rao<sup>2</sup>.

(4) Another type of variation observed was the occurrence of different flower, pod, and seed colours on the same plant. Yellow and orange red flowers were observed on different branches of the same plant.

### Discussion

Variants which first affect parts of the body other than the germ cells or germ nuclei are somatic variants; they may be propagated vegetatively or may be transmitted by sexual reproduction when reproductive cells are formed in the changed part.

In *Cajanus* activation of cotyledonary buds is environmentally conditioned; one of these axillary shoots was a variant totally different from the mother plant. As growth and differentiation progressed 'sports' for various characters like the determinate habit or variants for flower colour or

pod striations also occurred in various plants. All these somatic variants add to the 'apparent offtypes' and some of them could be of selective value if the characters affected are economically important. Since the frequency of occurrence of such variants is apparently high, they could be significant to pigeonpea improvement.

The variants observed could be mutational in origin, but mutations have a low probability of occurrence and are not expected to appear simultaneously in several cells of a tissue but the same is not true of treptions which may occur simultaneously in all or several cells of a tissue or region of the body as has been reported in maize, flax, *Antirrhinum majus*, etc. Treptions are the result of a natural stimulus which triggers some regulatory process whereas mutations result when a mutagen interferes with the regulatory mechanisms of the cells so that they do not work to completion<sup>3</sup>.

The *Cajanus* types Hy-3 A, Hy-3 B and Hy-3 C are sister lines, which are of economic worth isolated from a single accession 2817. Hy-3 A and Hy-3 B are similar in all respects except for seed colour; Hy-3 C has orange red flowers and more basal branches as against yellow flowers and lack of basal branching in Hy-3 A and Hy-3 B. Several other variants of this family are also under study. In spite of growing these populations in isolation and selecting plants true to type, further variation continues to manifest. The isolation of similar but slightly different sister lines and the occurrence of continued variation in spite of purification efforts cannot satisfactorily be explained on the basis of possible outcrossing only. At least some of the variants appear somatic in origin. Further studies on the origin and frequency of somatic variations and their role in the improvement of *Cajanus* are being investigated.

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## TWO FUNGAL PARASITES OF THE EGGS OF *CHANNA STRIATUS* (BL.)

ALTHOUGH several reports of fungal infections of fishes and fish eggs have appeared in literature from temperate and tropical regions, very little work so far has been done on fish mycopathology in India. The only report on fungal parasites of freshwater fishes of India is that of Bhargava *et al.*<sup>1</sup>.

During the course of an investigation on fungi associated with diseased fish and fish eggs, the authors collected eggs of *Channa striatus* in June 1974, from Ramgarh Tal, Gorakhpur. Many of the eggs had mycelial outgrowths, visible with naked eyes, on their surface. Detailed microscopic observations in the laboratory revealed that 70% of the eggs had the presence of fungal mycelium on them. The infected eggs were opaque and whitish in appearance while the healthy eggs were transparent and yellow in colour.

Isolations were made from the infected eggs on boiled hempseed-halves and unifungal, bacteria-free cultures were raised on the lines described by Raper<sup>2</sup>, Tiffney<sup>3</sup> and Johnson<sup>2</sup>.

These isolates were identified as *Aphanomyces laevis* and *Achlya flagellata* with the help of monographs of Johnson and Scott<sup>4</sup>.

In order to establish the pathogenicity of the isolates obtained, controlled infection investigations were conducted in the laboratory for each of the two isolates by standard methods. In the experiments conducted with *Aphanomyces laevis* fungal mycelium, protruding from the surface of all the eggs could be seen after 24 hours of the start of the infection test. The transparency of the contents of these eggs was changed to opaqueness. None of these eggs hatched. In the case of *Achlya flagellata*, the infection developed only on 60-70% of the eggs. The remaining 30-40% eggs hatched after the second day releasing hatchlings. Most of these hatchlings, however, subsequently developed fungus infection on their surface and died.

These observations showed that the eggs of *Channa striatus* are susceptible to the attack of *Aphanomyces laevis* and *Achlya flagellata* and that *Aphanomyces laevis* is a more virulent parasite of these eggs causing 100% mortality.

Out of these two parasites *Aphanomyces laevis* had been reported earlier as a parasite of the eggs of *Salmo gairdneri* by Scott<sup>5</sup>. The present report, however, is the first about the occurrence of this fungus on the eggs of *Channa striatus*.

Although *Achlya flagellata* has been reported earlier as a fish parasite by Tiffney and Wolf<sup>7</sup>, it has never been reported in literature as a parasite of fish eggs. The present communication, therefore,

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is the first report of *Achlya flagellata* as a parasite of fish eggs.

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#### <sup>14</sup>C INCORPORATION IN CHLORAMPHENICOL INHIBITED GROWTH AND ITS REVERSAL BY RIBOFLAVIN IN GREEN GRAM

SEEDS of *Phaseolus aureus* L. (cultivar) were germinated in sterilized petri dishes containing 20 PPM of riboflavin, 20 PPM of chloramphenicol and their combination in a luminosity of 1000 Lux and at 25°C ± 1°C. The presowing soaking treatment of riboflavin and chloramphenicol was given only for 24 hrs to avoid the possibility of infection with vitamin and then they were allowed to grow in distilled water. <sup>14</sup>C carbon dioxide was fed to the shoot system of the 7 day old seedlings by liberating <sup>14</sup>CO<sub>2</sub> by the action of 3 ml of 3N HCl on 25 µci of NaH <sup>14</sup>CO<sub>3</sub> in a <sup>14</sup>CO<sub>2</sub> feeding assembly for 10 minutes at a light intensity of 20,000 Lux. The samples were extracted with 80% alcohol. The alcohol insoluble residue was hydrolysed with a mixture of equal parts of boiling formic acid (77.5%) and 5 N HCl. The radioactivity of alcohol soluble and alcohol insoluble fractions were measured as per the method of Sinha<sup>1</sup> using end window GM Counter.

Basing on the earlier observation by Gopala Rao<sup>2</sup> that riboflavin increases chlorophyll synthesis, growth and protein content, the present study has been designed to ascertain whether riboflavin is involved in CO<sub>2</sub> fixation or not. The increase in protein content by riboflavin treatment and hence its capacity to reverse the inhibition of growth by chloramphenicol (inhibitor of protein synthesis) was reported earlier by Gopala Rao<sup>3</sup>. As chloramphenicol also inhibits chlorophyll

synthesis<sup>4</sup> it was felt necessary to study the interaction of chloramphenicol and riboflavin to assess the involvement of riboflavin in CO<sub>2</sub> fixation, related to photosynthesis at least indirectly.

TABLE I  
<sup>14</sup>C incorporation into alcohol soluble and insoluble fractions  
(Radioactivity expressed as counts/mt/g fresh wt.)

|                              | Alcohol soluble             |                     | Alcohol insoluble           |                     |
|------------------------------|-----------------------------|---------------------|-----------------------------|---------------------|
|                              | Radio-activity incorporated | % of radio-activity | Radio-activity incorporated | % of radio-activity |
| Control                      | 9,937                       | 100                 | 6,712                       | 100                 |
| Chloramphenicol              | 2,512                       | 25                  | 1,862                       | 28                  |
| Riboflavin                   | 14,325                      | 144                 | 8,512                       | 127                 |
| Riboflavin + Chloramphenicol | 8,237                       | 83                  | 3,712                       | 55                  |

In the present study <sup>14</sup>C fixation was more in the riboflavin treated seedlings (both in alcohol soluble and alcohol insoluble fractions) than in the control seedlings. <sup>14</sup>C incorporation was very low in chloramphenicol treated seedlings when compared to that of controls. The labelling was intermediate in the chloramphenicol and riboflavin combination. Apparently this may indicate that riboflavin can increase photosynthetic efficiency which can be substantiated by the earlier report that it increases chlorophyll synthesis. Biotin was known to be involved in CO<sub>2</sub> fixation<sup>5</sup>.

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#### CELLULOLYTIC ENZYME PRODUCTION/ NON-PRODUCTION BY SOME PATHOGENIC FUNGI

CELLULOLYTIC enzymes, though known since Ward's elucidation in 1888, are not of much consequence in pathogenesis. Nonetheless, they are important on account of their accessory role. Major portion of our knowledge of these enzymes is contributed

by scientists working with fibre and wood deterioration.

The potentials of five pathogenic fungi for the production of cellulase (Cx) has been examined. These are: *Curvularia lunata* var. *aeria* (leaf-spot of cotton); *Macrophomina phaseolina* (root-rot of *Sesamum*); *Nigrospora sphaerica* (leaf-spot of cotton); *Phytophthora parasitica* var. *sesami* (leaf-blight of *Sesamum*); and *Scopulariopsis brevicaulis* (fruit-rot of *Achras sapota*).

The enzyme was examined in 14 days old culture filtrates of the various fungi, grown on Richard's solution containing sucrose and Richard's solution containing Carboxymethyl cellulose (CMC). The enzyme was assayed for Cx activity viscometrically (by measuring loss of viscosity) and production of reducing sugars from carboxymethyl cellulose by DNS method (Miller, 1959).  $C_1$  enzyme has no effect on it. The results obtained are depicted in Table I.

TABLE I

| Fungus  | Cellulase activity* |          |
|---|---------------------|----------|
|   | Medium 1            | Medium 2 |
| <i>Curvularia lunata</i>                          | 0                   | 0        |
| <i>Macrophomina phaseolina</i>                    | 0                   | 50       |
| <i>Nigrospora sphaerica</i>                       | 0                   | 0        |
| <i>Phytophthora parasitica</i> var. <i>sesami</i> | 0                   | 20       |
| <i>Scopulariopsis brevicaulis</i>                 | 0                   | 0        |

\* Enzyme activity expressed as reciprocal of time for 50% loss in viscosity,  $\times 1,000$ .

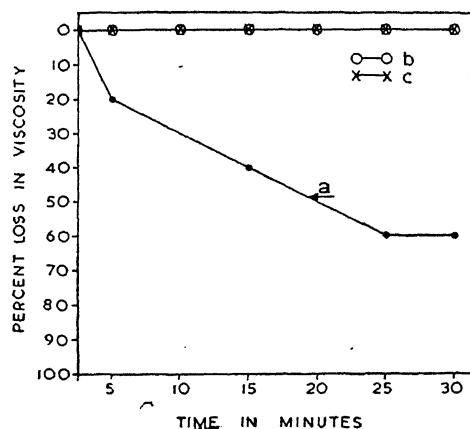


FIG. 1. Showing rate of loss in viscosity of CMC by 14 days old culture filtrate of *Macrophomina phaseolina* growing on (a) Richard's solution containing CMC, (b) Richard's solution containing sucrose, and (c) boiled culture filtrates.

As is evident, there was no cellulase activity in the culture filtrates of the organisms growing on

sucrose as the exclusive carbon source. Cellulase activity was present only in the culture filtrate of *M. phaseolina* growing on Richard's CMC medium. The enzyme sample reduced the viscosity of CMC by 50% in 20 minutes (Fig. 1). No cellulase enzyme activity could be detected in culture filtrate of the organism, growing on sucrose.

None of the other fungi could reduce the viscosity by 50% even in extended incubation periods, but for *Phytophthora parasitica* in which the 50% loss in viscosity was recorded in 150 minutes. Liberation of reducing sugars was undetectable in the DNS method.

All these fungi, in another series of experiments, invariably, produced pectic enzymes on different media, Gour (1974). It is inferred, therefore, that cellulytic enzymes are not so commonly produced by fungi.

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## NATURAL OCCURRENCE OF MILKY DISEASE BACTERIUM, *BACILLUS POPILLIAE* DUTKY ON WHITE GRUBS IN INDIA

SUGARCANE white-grubs assumed pest proportion in the farm at the Sugarcane Breeding Institute, Coimbatore, and its neighbourhood towards late sixties. Detailed investigations revealed the presence of a disease in the white-grub under field conditions, in and around Coimbatore. This has now been identified as a strain of *Bacillus popilliae* Dutky by Dr. H. Tashiro. A similar strain of the disease is also reported to occur in Gujarat on *H. consanguinea* Bl. (Talati, 1973).

Periodic examination of the grub population in the farm at this Institute showed (Table I) that the parasitisation of the grub by the bacterium varied from 2.2 to 17.9% during July 1973 to February 1974, the period when the grub and the bacterium are available in the field. The parasitisation was low when the grubs began their feeding on sugarcane roots but went up appreciably by November.

TABLE I

Natural incidence of the strain of *Bacillus popilliae* on *H. serrata* at Coimbatore

| Sl. No. | Month     | % parasitisation of grubs | Range of soil temperature at 15 cm below soil surface in °C | Rainfall in mm |
|---------|-----------|---------------------------|---|----------------|
| 1973    |           |                           |   |                |
| 1.      | July      | 2.7                       | 23.0 to 34.5  | 74.4           |
| 2.      | August    | 2.2                       | 23.5 to 34.5  | 22.9           |
| 3.      | September | 4.0                       | 28.0 to 36.5  | 20.8           |
| 4.      | October   | 7.5                       | 22.0 to 35.0  | 265.6          |
| 5.      | November  | 10.5                      | 22.0 to 32.0  | 57.6           |
| 6.      | December  | 17.9                      | 21.0 to 31.5  | 135.0          |
| 1974    |           |                           |   |                |
| 7.      | January   | 15.4                      | 21.5 to 31.0  | Nil            |
| 8.      | February  | 16.6                      | 21.0 to 35.5  | Nil            |

It appears that the temperature range of 21° to 32° C favours bacterial multiplication and the infection of the grub.

This strain of bacterium was easily multiplied in the laboratory on the natural host by inoculating the healthy grubs with 1 million spores per grub using an 'Aglia' microsyringe. The infected grubs exhibited typical milky white appearance in about 16 to 20 days (Fig. 1), but the entire body became



FIG. 1. Infected grub *H. serrata* showing milky white appearance.

white in about 30 to 50 days. Subsequently the grubs stopped feeding, became inactive and the body shrivelled up, leading to gradual death in about 2 months, after infection. In the laboratory, under artificial inoculation, the grubs of *H. serrata* and *Anomala bengalensis* Blanch were parasitised

to the extent of 67 and 72%. Dr. Tashiro (personal communication) mentions that he multiplied this bacterium on *Amphimallon majalis* Razoumowsky and *Popillia japonica* Newman.

Subsequently different methods of grub infection in soil were tested by inoculation of spore suspension in potted sterile soil with sugarcane plants and healthy grubs (Table II).

TABLE II

Efficacy of different methods of inoculation of bacteria—in soil

| Treatment   | Strength (Spore load)                          | Total grubs inoculated and survived | % infection |
|---|--|-------------------------------------|-------------|
| Feeding healthy grubs in a solution of bacterium for 15 minutes | 1 million spores per ml                        | 43                                  | 53.5        |
| Soil inoculation with infected grubs after macerating them      | 1 million spores per ml—500 ml in 4 kg of soil | 48                                  | 56.3        |
| Pouring suspension of the bacteria                              | do.  | 44                                  | 52.3        |
| Sterilized roots dipped in spore suspension for 15 minutes      | 1 million spores per ml                        | 39                                  | 61.5        |
| Control   | 500 ml of sterile water                        | 19                                  | 0.6         |

Test indicated that infection of grubs occurred through ingestion of the bacterium, present either on the roots or in the soil around the root-zone. The disease symptom in the grubs, namely, milky white appearance, appeared in 30 to 60 days. Highest infection of grubs occurred, when roots dipped in spore suspension were fed. Satisfactory infection was also induced in pots where inoculation was done by macerating the grubs and also where spore suspension was poured on the grubs of the soil. The results indicate that the grubs can be infected under field conditions if sufficient population of the bacterium is introduced in the soil.

We express our gratitude to Dr. H. Tashiro, Professor, New York State Agricultural Experiment Station, Geneva, New York, for kindly identifying the bacterium and to the Director, Sugarcane Breeding Institute, for facilities provided.

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# SCLEROTIUM WILT OF PSORALEA CORYLIFOLIA

*Psoralea corylifolia* L. is an annual medicinal plant found throughout India<sup>2</sup>. A sclerotium wilt was noticed during December, 1969, in the Ayurvedic Garden of the Banaras Hindu University. Dull appearance of the infected plants at maturity marks the appearance of disease symptoms. Later lower leaves become yellow followed by wilting and drying up. Ultimately the whole plant wilts. Creamy white mycelium of the fungus was observed over and around the roots in the soil. Globulate, yellowish brown sclerotia, 0.5–1.5 mm in diameter developed on the infected plant roots in large numbers. Under high humidity conditions infected roots exhibit rotting symptoms.

The fungus was identified as *Sclerotium rolfsii* Sacc. (Fig. 1). Pathogenicity was proved under

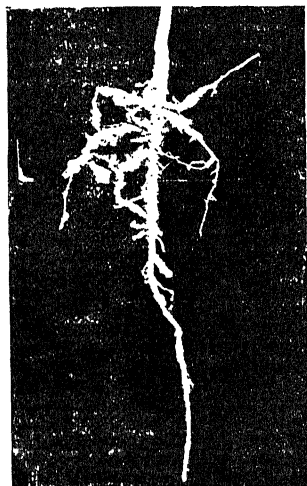


FIG. 1. Infected root of *Psoralea corylifolia*.

sterilized conditions on one month old plants raised in pots and using sclerotia produced on PDA as inoculum. Reisolates from artificially infected plants were similar to the original isolates.

*Cercospora latens* Ell. and Ev.<sup>3</sup>, *Phyllosticta psoraleae* (Cooke) Tassi<sup>1,5</sup> and *Colletotrichum corylifolia* Pavgi and Singh<sup>4</sup> are the only recorded parasitic fungi from India on *P. corylifolia*.

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## ROOT DORMANCY IN POLYALTHIA LONGIFOLIA BENIH. AND HF.

TIPS of dormant roots are often opaque brown, while tips of growing roots are apt to be white or lightly coloured. Studies by Müller (1906) and others suggested that the apical meristems of dormant roots are isolated from outside by layers of cells with lignified and suberised walls. The formation of these layers was referred to as "Metacutisierung" by Plaut (1910). Wilcox (1954) used the anglicised form "metacutization" which will be used here. Plaut (1918) described four distinct types of metacutization in gymnosperms.

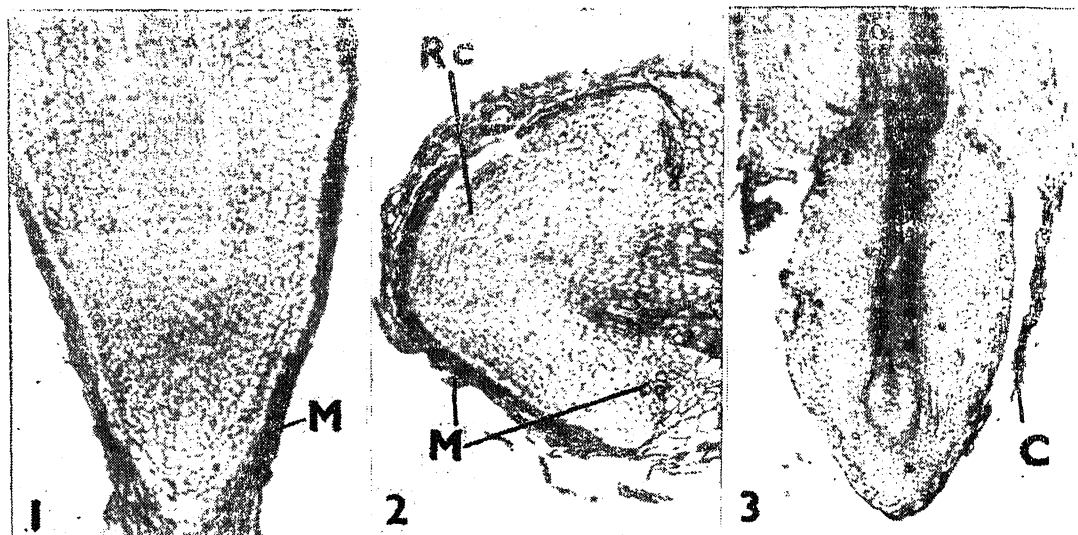
This report concerns with two types of metacutization exhibited by roots of *Polyalthia longifolia* collected from the same plant on the 6th November, 1974, when the winter was just beginning. The root apical meristem shows a central group of common initials for all the root zones. The types of metacutization observed are :

I. The outer cell layers of the root cap show metacutization. Four to seven outermost cell layers of the root body also get metacutized which get connected with the metacutized root cap. The root apex is thus completely covered by metacutis (Fig. 1). This is similar to Plaut's Type I and has been reported in some cycads, dicotyledons and some *Pinus* species (Romberger, 1963).

II. In addition to the metacutized outer layers as in I, some cells of the cortex proximal to the root initials also show metacutization, as also the endodermis. The metacutized endodermis gets linked with the metacutized cells of the cortex and the cap so as to cover the root initials like a thimble (Fig. 2). This resembles Plaut's Type III mainly reported in conifers.

When the root breaks dormancy, the metacutized cell layers are broken through leaving a collar-like structure around the root (Fig. 3).

Pillai (1963, 1964) reported metacutization in some cycad and conifer roots. There are very few reports of this phenomenon in Angiosperms. The occurrence of 2 types of metacutization in roots of the same plant questions the advisability of rigid typification. Scott (1928) and Hayward and



FIGS. 1-3

Blair (1942) interpreted metacutization as an effect of adverse environmental conditions. Pillai (1964) suggested that environmental factors alone may not be responsible because the same plant bears active and metacutized roots at the same time. This holds good in the present report also. Moreover, the collection was made in early November, when the temperature and soil moisture were still favourable for growth. It is to be concluded that there is little synchrony between different parts of the same root system as regards the induction or breaking of root dormancy. Wilcox's (1954) suggestion of changes in the root auxin content causing dormancy points out possible lines for experimentation. Wilcox (1962) suggested that a number of hormonal factors may be operating. Any explanation based on hormonal control has evidently to assume a considerable degree of autonomy to the individual root tips. The physiological mechanisms controlling root dormancy are still not well understood and the control system does not appear to be a simple one.

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University of Rajasthan,  
Jaipur, May 14, 1975.

AMBUJA PILLAI.  
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\* Not seen in original.

#### ABNORMALITIES IN THE DEVELOPMENT OF MALE GAMETOPHYTE IN *MORUS* *LAEVIGATA*

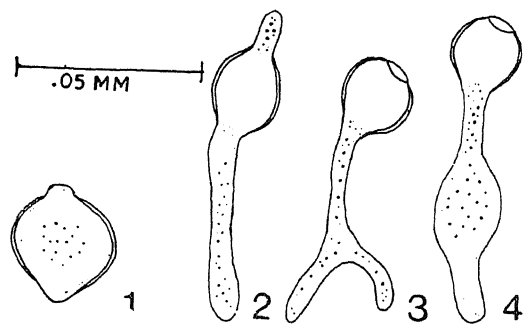
THE male gametophyte of angiosperms is generally considered as a monesiphonous, unbranched structure containing two sperms preceded by a tube nucleus<sup>2</sup>. Although pollen grains of many taxa of angiosperms have several germ pores, one pollen tube develops from each pollen grain. However, in some angiosperm families, such as Malvaceae, Cucurbitaceae and Campanulaceae and in *Clarkia* regular occurrence of polysiphonous condition is reported.<sup>2,3</sup> A branching of the pollen tube has also been observed sometimes as in some Amentiferae<sup>3</sup>, *Clarkia* and *Lilium*<sup>2</sup>. Such an abnormality is specially common after colchicine treatment. The branching of pollen tube can also be induced by the addition of 2, 4-D or pollen extract to the medium<sup>4</sup>.

The author, while studying the development of the male gametophyte in *Morus laevigata* Wall. has observed the occurrence of polysiphonous pollen grains and branching of pollen tubes in this taxon. As these abnormalities are not so far reported in Moraceae, this report deals with the same.

The pollen grains of *Morus laevigata* have three germ pores. They swell noticeably by absorption

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of water prior to the development of pollen tube. Usually one pollen tube grows from a germ pore but occasionally a second pollen tube may also develop from another germ pore (Fig. 1). Although both pollen tubes emerge at the same time, one of them grows faster and shows dominance over the other (Fig. 2). However, in *Clarkia Johnston*<sup>2</sup> reported that both the pollen tubes grow almost at the same rate. The pollen tubes also show occasional branching (Fig. 3). In a few cases the pollen tube enlarges into



FIGS. 1-4. *Morus laevigata*. Fig. 1. A pollen showing emergence of two pollen tubes. Fig. 2. A pollen with two pollen tubes of unequal size. Fig. 3. A pollen with a branched pollen tube. Fig. 4. A pollen tube enlarge into a bladder-like structure.

a bladder-like structure (Fig. 4). Such structures are sometimes described as "secondary pollen grains"<sup>1</sup>. Certain globule-like structures, green in colour were observed in the pollen grains and pollen tubes. These structures gave a starch-negative test.

The author is thankful to Dr. V. Singh for valuable guidance and to the State C.S.I.R., Lucknow, for financial assistance.

School of Plant Morphology,  
Meerut College,  
Meerut, May 9, 1975.

SATISH KUMAR.

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# A NEW FRUIT ROT OF BRINJAL (*SOLANUM MELONGENA* L.) CAUSED BY *CORYNESPORA CASSICOLA* (BERK. AND CURT.) Wei.

DURING March to June, 1973 and 1974 fruits of brinjal (Pusa Kranti and Pusa Purple Long) showed severe rot symptoms as small, brown depressed spots with violet margin in the experimental plots of H.R.C. Patherchatta, Pantnagar (Nainital). In presence of high humidity and moisture the spots enlarge and coalesce together. Such spots are usually 1.5-2.5 mm deep. Later, centre of the spot turns grey on which white fungal growth appears. In dry weather, development of the spots is restricted (Fig. 1). In certain brinjal varieties 30-40% of the fruit is damaged.

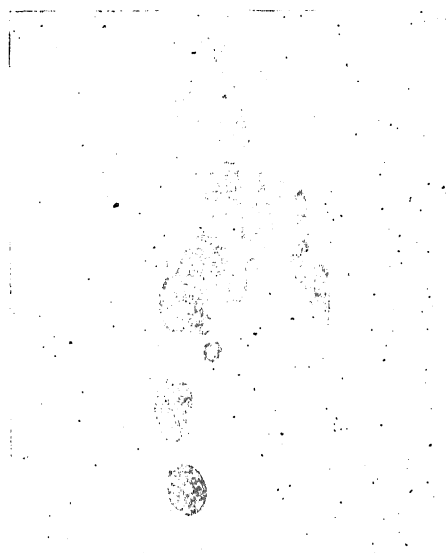


FIG. 1. Brinjal fruit affected with *C. cassicola*.

Microscopic examination revealed that the mycelium of the fungus was septate and dark brown in colour from which arose conidiophores, mostly single but occasionally in group. These were perpendicular to the surface of the substratum, unbranched, straight cylindrical, light coloured with a prominent bulbous base, septate (4-12 septa), slightly swollen at the tip and measured  $60.5-210.0 \times 4.5-6.0 \mu$  (Fig. 2).

The fungus was isolated on potato dextrose agar (PDA) by single spore isolation. Pathogenicity of the fungus was confirmed by putting a small amount of culture on injured fruits. Uninjured fruits inoculated with the fungus did not produce spots. The fungus on artificial inoculation to vegetative



parts of the brinjal by the method as described by Asha Ram and Lele<sup>2</sup> for pathogenicity test failed to cause infection.

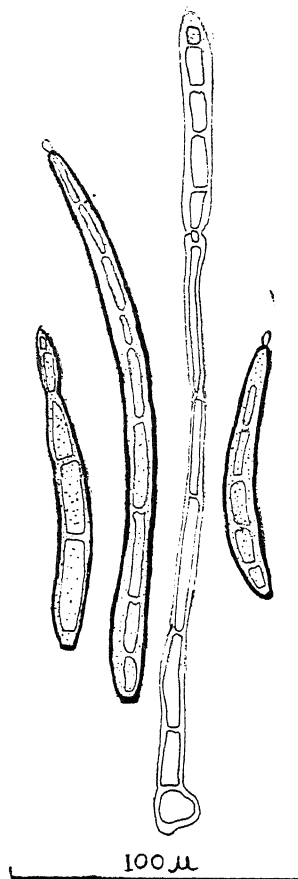


FIG. 2. *Corynespora cassicola*; Conidia and conidiophore.

On artificial inoculation, the fungus infected fruits of 36 varieties of brinjal and tomato but could not infect fruits of banana, apple, orange, citrus, pea pod, potato tuber and carrot root.

The seeds from naturally rotten fruits of brinjal variety Pusa Kranti were collected and isolations made from seed coat, embryo and endosperm by standard methods revealed the absence of the fungus in the seed.

The fungus studied herein on the basis of morphological and taxonomic characters as described by Wei<sup>5</sup> was identified as *Corynespora cassicola* (Berk. and Curt.) Wei, culture of which has been deposited at CMI, England (IMI 182694). The fungus has been recorded for the first time causing

fruit rot of brinjal. Earlier the fungus was recorded on fruits of tomato, cucumber and papaya (Wei<sup>5</sup>) and caused leaf spot of brinjal but not fruit rot (Asha Ram and Lele<sup>2</sup>). Since the present isolate did not produce any leaf spot on brinjal it may be a different strain of *C. cassicola* described by Asha Ram and Lele<sup>2</sup>. Agarwal<sup>1</sup>, Munjal and Gill<sup>3</sup>, Thirumalachar and Lacy<sup>4</sup> and many others have also reported this fungus on several other plants in India.

Since the disease is soil-borne, a three years crop rotation with non-susceptible host may be followed to prevent the disease.

Thanks are due to Director, CMI, England, for confirming identification of the fungus.

Department of Botany and G. S. DUBEY,

Plant Pathology,  
Himachal Pradesh University,  
Agricultural Complex, Solan (H.P.),  
June 14, 1975.

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#### EVOLVING BREEDING SYSTEM IN RAI (*BRASSICA JUNCEA*)

RAI (*Brassica juncea*) is a predominantly self-pollinating oilseed crop. Its low recombination potential resulting from the self-fertilizing system of mating is a major deterrent limiting the speed with which desirable genetic materials from diverse sources could be brought together, whether for heterosis breeding or for recombination breeding. Any biological device, therefore, promoting outbreeding will have an important bearing on the breeding efficiency of self-pollinating crops. This note is to report on an Advanced Stigma mutant (a device promoting outbreeding) that we isolated in 1974-75 season in an appressed pod accession of rai germplasm collection.

There was substantial intra-plant variation in the degree of stigma advancement (Fig. 1). The Advancement Index (ratio of pistil length above corolla tip/corolla length) ranged from 0.15 to as high as 0.71, with a mean value of 0.503. Unlike normal plants, the flower buds of the mutant displaying protruding stigma in the evening were found as such the next morning, or, if the buds had opened into flowers, corolla and anthers held

the lower levels than their corresponding stigmas. Occasionally, it was observed that the fertilization had taken place; the silique development had started but anthers of that flower had not dehisced yet (Fig. 1). All the flower buds with advanced stigma did not necessarily open into flowers the next day: the time taken for flower opening in some cases was as high as four additional days. Also the flowering sequence in some of the inflorescences did not strictly follow the normal pattern of acropetal succession. The advanced stigma plant was presumably late in flowering and matured three weeks later than the normal plants of the accession.

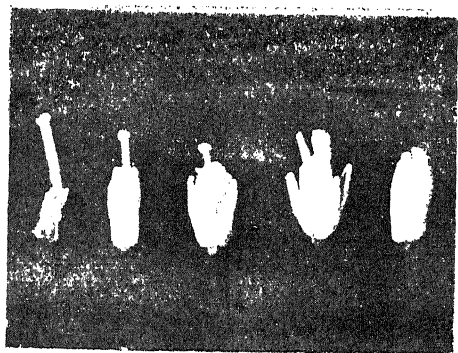


FIG. 1. Intra-plant variation in the degree of stigma advancement. The leftmost flower with corolla removed shows the failure of anthers dehiscence even after the fertilization has taken place.

Unlike the advanced stigma mutants reported by Singh<sup>1</sup>, and Asthana and Singh<sup>2</sup> where all the flowers uniformly displayed advanced stigmas, the frequency of advanced stigma flowers in this mutant was approximately 67%, which cannot ensure the production of pure hybrid seeds. However, it may foster composite breeding to upgrade the populations for complex traits. In essence, this morphological device would work like the physiological system of partial male sterility reported in barley<sup>3,4</sup>.

Department of Genetics and  
Plant Breeding,  
Banaras Hindu University,  
Varanasi, May 27, 1975.

K. N. RAI.  
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## A NEW SPECIES OF *AMORPHOIDEA* (CURCULIONIDAE, COLEOPTERA) INFESTING COCONUT INFLORESCENCE

DURING November 1973 small weevils of the genus *Amorphoidea* were found in large numbers on the inflorescence of coconut palms in Coimbatore and adjoining areas. The infestation is generally noticed as dark areas at the points of attachment of the lower spikelets on the central axis of the inflorescence and the grubs develop in such infested patches. The weevil is also seen in large numbers in the affected parts and among the male flowers on the inflorescence. Though slight reduction in the nut yield is attributed to the infestation of the weevil by the growers, yet, it does not appear to be a pest of importance on coconut in South India.

So far, no species of *Amorphoidea* has been reported from India infesting coconut inflorescence. However, Ekanayake<sup>2</sup> reported the association of a species of *Amorphoidea* with the inflorescence of coconut in Sri Lanka. Dr. R. T. Thompson, of the British Museum (Natural History), London, in his personal communication pointed out that the specimens of *Amorphoidea* from coconut inflorescence may relate to specimens from Sri Lanka determined by him in 1963 and further confirmed that the species has not been described. In this paper the new species has been described under the name *Amorphoidea coimbatorensis*.

*Amorphoidea coimbatorensis* sp. nov.

Testaceous brown, covered with very short recumbent golden setae.

### Male

**Rostrum** : slender and 0.46 mm long, shorter than pronotum, gently curved, very gradually widening from base to apex; dorsum not flattened and with faint longitudinal striae with confluent punctures. **Antennae** : testaceous brown inserted beyond the middle of the rostrum. **Prothorax** : transverse, 0.63 mm long and 0.46 mm wide, gradually narrowing from base and sharply constricted towards the anterior portion; distinctly constricted at the apex, sub-truncate at the base and apex, the dorsum gently convex longitudinally, with dense fine shallow sub-reticulate punctures throughout. **Elytra** : broadly ovate, with well marked closely punctate striae, the intervals much broader than striae, finely rugulose. **Legs** : devoid of any tooth, all femora expanded, apex of tibiae and tarsi densely hairy. **Sternum** : elevated.

**Genitalia** (Fig. 1) : **Penis** : elongate, chitinized, slightly concave on the dorsal side, twice as long as wide, sides sub-parallel up to apical margin, apical margin gradually sloping and depressed.

Sloping margins on both sides bear setae, convex on lateral view, dorsal area membranous. *Tegmen* : basal piece forming a ring round the penis, dorsal margin thickened in the middle. *Parameres* : absent, manubrium short, broader and chitinized at the anterior end, tip slightly pointed. *Internal sac* : short, distal half slightly pigmented with armature of elongated scales, granulated scales, other area with number of papillae; internal sac valve placed widely apart. *Apophyses* : shorter than penis well chitinized, curved, proximal tip rounded. *Spicule* : medium sized well chitinized, more or less straight, about three times as thick as apophyses, proximal end slightly curved and pointed, prongs wide apart.

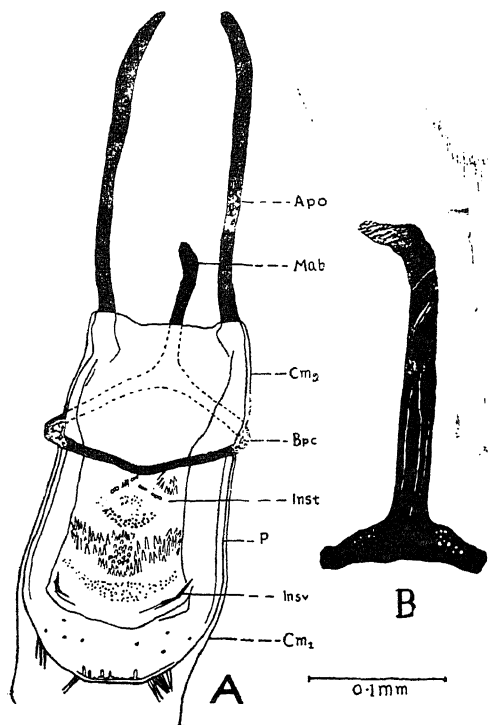


FIG. 1. Male genitalia of *Amorphoidea coimbatorensis* sp. n. P, Penis; Bpc, Basal piece; Mab, Manubrium; Inst, Internal sac; Apo, Apophyses; Insv, Internal sac valve; Cm<sub>1</sub>, First connecting membrane; Cm<sub>2</sub>, Second connecting membrane; A, Dorsal View; B, Spicule.

### Female

*Rostrum* : slender, 0.63 mm long, slightly shorter than pronotum, more curved, very gradually widening from base to apex. *Dorsum* : not flattened and with faint longitudinal striae with confluent punctures. *Antennae* : testaceous brown, inserted at the middle of the rostrum. *Prothorax* : with apical constriction. *Sternum* : elevated.

*Length* : Male 2.16 mm, female 2.33 mm.

*Breadth* : Male 0.78 mm, female 0.82–0.91 mm.

This species differs distinctly from the commonly noticed species *Amorphoides arcuata* Motsch in South India on cotton flowers<sup>1</sup>. *A. arcuata* is characterised by possession of femoral tooth on legs and the male genitalia with the apical margin of penis being flattened and spatulate and the spicule curved on both sides at its proximal end. The new species differs from the above species in lacking the femoral tooth, the apical margin of penis being gradually sloping and depressed and the proximal end of the spicule slightly curved and pointed.

*Material Examined* : India, Coimbatore, 10 males, 15 females, from coconut inflorescence, 17–11–1973, B. V. David.

Holotype male and Allotype female in the collection of the Department of Entomology, Tamil Nadu Agricultural University, Coimbatore. Paratypes deposited in the collections of the Zoological Survey of India, Calcutta, the Division of Entomology, Indian Agricultural Research Institute, New Delhi, and the British Museum (Natural History), London.

The authors wish to express their sincere thanks to Dr. R. T. Thompson, British Museum (Natural History), London, for having kindly furnished the necessary information on species of *Amorphoidea*.

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B. VASANTHARAJ DAVID,  
Coimbatore 641 003,  
June 20, 1975.

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## SHORT SCIENTIFIC NOTES

### Crystallographic Data of DL-Leucyl-glycyl-glycine

As a part of a programme to study crystal structure and conformation of simple peptides, we have undertaken structure analysis of the tripeptide DL-Leucyl-glycyl-glycine ( $C_{10}N_3O_4H_{17}$ ).

Long needle-shaped crystals of DL-leucyl-glycyl-glycine were obtained from an aqueous solution of the substance by slow evaporation. The unit cell dimensions and the space group were determined from oscillation and Weissenberg photographs taken about crystallographic axes using  $CuK\alpha$  radiation. The crystal data: (1) crystal system-monoclinic. Lattice parameters,  $a = 12.18$ ,  $b = 11.43$ ,  $c = 9.64$  Å,  $\beta = 104.50^\circ$ , density (measured)—1.250, density (Calc)—1.255, Molecules per unit cell  $Z = 4$ , Systematic absences  $OkO: k = 2n + 1$ ,  $hol: l = 2n + 1$ , Space group  $P2_1/c$  (No. 14).

Three-dimensional intensity data have been collected and the structure analysis is in progress.

Neutron Physics Section, K. N. GOSWAMI.\*  
B.A.R.C., Bombay-85, V. S. YADAVA.  
July 24, 1975.

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### Record of Lunate Fly, *Eumerus* sp. (Syrphidae : Diptera) on Potatoes from India

While harvesting potatoes (September, 1974) at Central Potato Research Institute, Simla, some tubers were found infested with maggots which were reared in the laboratory. Flies emerged from these tubers after about 15 days and these were identified as species of *Eumerus*.

Lunate flies (lesser bulb flies) belonging to genus *Eumerus* are well distributed over many parts of the world<sup>1</sup> and these infest a variety of bulbs<sup>2-3</sup> and tubers including potato<sup>4</sup>. In Germany 5% loss in potato crop has been reported by this fly<sup>5</sup>. The infested portions of the plants consist of slimy decayed tissues and usually infested with diseases like bacterial rot<sup>6</sup>, basal rot, *Fusarium*<sup>7</sup>, etc. From the existing literature on insect pests of potato crop, it seems that *Eumerus* sp. has not so far been reported from India.

The authors are grateful to Mr. K. M. Harris (Dipterist) of British Museum Natural History, London, for identifying the flies.

Division of Entomology,  
Central Potato Research  
Institute,

Simla 171 001, (H.P.), July 30, 1975.

S. S. MISRA.

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### Aneuploids in *Trigonella foenum-graecum*

The fodder and condiment crop *Trigonella foenum-graecum* L. has the diploid chromosome number  $2n = 16$  and the artificial autotetraploids, developed in this university has the  $4n$  number 32. Three autotetraploid strains are superior in vegetative yield compared to the diploids and other autotetraploid strains. This note reports on the occurrence of aneuploids in the autotetraploid populations.

The pollen mother cell analysis was done on the floral buds fixed in acetic alcohol (1:3) and squashed in acetocarmine. Pollen fertility was based on stainability with acetocarmine.

Thirteen hypertetraploid plants were found in the autotetraploid populations in the years 1972-73 and 1973-74. The hypertetraploids were shorter than the diploid and the autotetraploid plants and had whitish and drooping leaves. They had internodes almost equal to those of the diploids but fewer than those of the autotetraploids. They had fewer branches, shorter leaves and pods and fewer seeds per pod than the diploids and the autotetraploids.

The chromosome number in the hypertetraploids ranged from 33 to 36. The pollen mother cells showed different chromosomal associations at metaphase-I (Table 1). Configurations higher than the quadrivalent were not observed. The multivalent frequency of the hypertetraploids was higher than that of the autotetraploids. Distribution of chromosomes at anaphase-I was irregular and showed laggards. Pollen grains of the hypertetraploids and the autotetraploids were almost equal in size. Pollen fertility of different plants ranged from 48% to 92%.

TABLE I  
Chromosome associations at metaphase-I

| Chromosome No. | No. of Plants | Quadrivalents |      | Trivalents |      | Bivalents |       | Univalents |      | Average pollen fertility (%) |
|----------------|---------------|---------------|------|------------|------|-----------|-------|------------|------|------------------------------|
|                |               | Range         | Mean | Range      | Mean | Range     | Mean  | Range      | Mean |                              |
| 33             | 5             | 0-5           | 1.63 | 0-3        | 1.19 | 4-14      | 10.87 | 0-4        | 1.87 | 63.68                        |
| 34             | 4             | 0-4           | 1.16 | 0-2        | 0.97 | 7-15      | 11.62 | 0-5        | 3.09 | 61.69                        |
| 35             | 2             | 0-4           | 1.24 | 0-2        | 1.18 | 8-16      | 12.04 | 0-5        | 2.43 | 78.16                        |
| 36             | 2             | 0-3           | 1.21 | 0-4        | 1.95 | 9-15      | 11.75 | 0-4        | 1.82 | 70.16                        |

The number of seeds per plant was one-third of those of autotetraploids.

Though one or more chromosomes were present, more than four times in each hypertetraploid plant, no pentavalent or hexavalent was observed. This indicate that the higher polyploids may form only bivalents and that spatial factor also has some influence on pairing in a crowded nucleus which does not enlarge proportionate to the increase in chromosome number. In a 7 x plant of *T. corniculata* also, no multivalent higher than a quadrivalents was observed<sup>1</sup>. Anaphase-I irregularities in the autotetraploids result in the production of aneuploids. Though there was a significant reduction in the multivalent formation in the autotetraploids in C<sub>4</sub> and C<sub>5</sub> generations, irregular disjunction was still observed to some extent<sup>2</sup>.

Aneuploids are usually morphologically inferior to the diploids and the autotetraploids, and, are meiotically unstable. Thus they adversely affect the vegetative and seed yield of the autotetraploids. However, because of their low frequency, the aneuploids do not pose a serious problem to the autotetraploids of *T. foenum-graecum* because only seven such plants were found in half an acre area in 1973-74. They produce fewer seeds per plant and their influence on yield is not much. Nevertheless, they must not be present in the autotetraploids and since they are identifiable from morphological characteristics, they can be removed from the autotetraploid populations. In rye which is grown for seed, aneuploids have been reported to greatly affect fertility of autotetraploids<sup>3</sup>. Similarly, in autotetraploid red clover upto 10% aneuploids were found<sup>4</sup>. The frequency of aneuploids in autotetraploids of *T. foenum-graecum* is expected to decline after further selection.

We are grateful to Dr. J. L. Minocha, for his interest in the work and suggestions. Financial assistance by the Indian National Science Academy, is thankfully acknowledged.

Department of Genetics,  
Punjab Agricultural University,  
Ludhiana, July 21, 1975.

AVTAR SINGH.  
DALBAR SINGH.

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#### Mercury in Fish from Mettur Reservoir

In recent years aquatic environmental pollution due to mercury has become a serious health hazard. Considerable work has been done on the determination of mercury in fish flesh. Matida and Kumada<sup>1</sup> have worked on the distribution of mercury in water, bottom mud and aquatic organisms of Minamata Bay, the river Agano and other water bodies in Japan.

A preliminary investigation was carried out on the incidence of mercury in fish from Mettur Reservoir of the Cauvery river system. Fish samples were collected from the catches of Mettur reservoir, sufficiently iced and brought by thermocole insulated boxes to the laboratory at Coimbatore, preserved in freezer chest and analysed the next day. The mercury in the fish samples was determined according to the method described by Nabrzyski<sup>2</sup>. The results are expressed on wet weight basis.

|                              | Total length (mm) | Weight (gm) | Mercury (ppm) |
|------------------------------|-------------------|-------------|---------------|
| <i>Mystus aor</i>            | 370               | 350         | 0.250         |
| <i>Notopterus notopterus</i> | 340               | 270         | 0.086         |
| <i>Cirrhina cirrhosa</i>     | 335               | 450         | 0.047         |
| <i>Labeo calbasu</i>         | 410               | 720         | 0.090         |
| <i>Rhinomugil corsula</i>    | 330               | 275         | 0.085         |
| <i>Puntius sarana</i>        | 255               | 250         | 0.072         |
| <i>Labeo rohita</i>          | 430               | 1570        | 0.027         |

The maximum permissible level of mercury in foodstuffs as laid down by U.S. Federal Legal Action guidelines is 0.5 ppm.

Our thanks are due to Tamil Nadu State Fisheries Department for suggesting this problem and also for providing the necessary facilities for collection of samples. We also wish to express our sincere thanks to Professor N. V. Choodamani, for the encouragement and for the keen interest he evinced in the course of the work.

Department of Fishery Science,  
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Coimbatore 641 003, September 12, 1975.

P. JEYACHANDRAN.  
SAMUEL PAUL RAJ.

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## REVIEWS AND NOTICES OF BOOKS

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**Introduction to Solid State Physics.** By C. Kittel. (Fourth Edition, Second Wiley Eastern Reprint), 1974. Pp. xvii + 766. Price Rs. 30.00.

By now the name of Kittel is a by-word in the Solid State Physics Departments all over the world and the book is becoming bulkier each edition. It is good to see that a fairly good reproduction of the fourth edition is coming out for a second reprint which shows the popularity of the book. At rupees thirty it is a good buy. The book is profusely illustrated and the photographs have not suffered too badly in this reprint. One hopes that with reasonably cheap reprints like the present one, students will develop the habit of acquiring a library of their own in the fields of their choice.

G. SURYAN.

**Cours et Documents de Mathematiques et de Physique.** Edited by Bruno Morando. (Gordon and Breach Science Pub. Ltd., 41/42, William IV Street, London, W.C. 2), 1975. Pp. xiv + 255. Price: Hardback \$12.80, Paper back \$4.80.

This book essentially deals with the motion of an artificial satellite about the earth. The study of the motion of earth satellite and their trajectory computation has become an extremely important subject during the last two decades. A significant feature of this book is that it develops the subject-matter step by step from the basic fundamentals and this is most helpful to a beginner in this field. Wherever possible, the basic theorems required to understand the mathematical formulations have also been discussed to enable the reader to follow the subject-matter easily.

The book is divided into 14 chapters. Motion of the satellite has been essentially dealt as a two body problem. The earth's oblateness which is the major perturbing force on the movement of the

satellite is discussed in detail. The basic concept of perturbations due to the solar and lunar gravitational attraction, atmospheric drag and radiation pressure on the motion of the satellite are treated in the last chapter. In addition, special problems like the problem of critical inclination and problem of resonance related to geostationary satellites are also explained in detail.

In order to facilitate easy understanding of the subject, the author has adopted Newtonian dynamical description which is familiar to most of the physics students. Expressions for various harmonics related to the perturbing forces, Laplacian coefficients and earth's potential are derived from fundamentals. Legendre polynomials and Bessel functions which form the backbone of celestial mechanics are discussed in detail as a prelude to an easy understanding of the expressions derived for describing the earth's potential and various perturbations. The perturbations due to the zonal and tesseral harmonics are also treated separately in the book.

The extensive references sighted in the bibliography as well as a large number of elegant figures included in the text should go a long way in making this book a very useful one to the students of Astrodynamics and celestial mechanics. A simple and elegant treatment of the subject-matter will also be very useful to the specialist in orbital mechanics, geodesy and geophysics.

U. R. RAO.

**Soil Biochemistry** (Vols. 3 and 4, Eds. E. A. Paul and Douglas McLaren (Marcel Dekker, Inc., New York), 1975. Pp. 334 and 277. Price \$27.50 and \$23.75 respectively.

In Vol. 3, there are six Chapters. Chapter 1 on Biochemistry of the Soil Subsystem deals with Plant-microbe and Soil-microbe relationships and

also the role of soil enzymes and measurement of microbial activity in soil. Chapter 2 gives in a simplified manner the complex subject of water and biogeochemical cycles and energy flow. Chapter 3 is confined to a relatively less understood area of Soil Microbiology, viz., Microbiology of flooded soils. The author has done a good job of covering the subject, though he has overlooked the pioneering work done in India on the subject. Chapter 4 covers another complex subject of Biochemistry and Microbiology of peats. Much remains to be understood on the subject, but the authors have presented the available information in a commendable manner. Chapters 5 and 6 are more general in nature and would be of general interest to Soil Scientists and Microbiologists.

In Vol. 4 there are six chapters. All the six chapters are of topical interest to biochemists and microbiologists. The first chapter on Nitrogen Transfer in Ecosystems has brought together valuable data from different sources to illustrate the importance of nitrogen in our ecosystem. Chapter 2 on Biochemistry of phosphorus deals with a less understood branch, but the author has done a good job of pooling the available information, and in summarizing the role of soil organic phosphorus in the Phosphorus Cycle in nature. Chapter 3 deals with the hydroxamic acids in soils, bringing out their roles in plant growth. The information presented gives ample scope for exploiting the natural processes in soil for boosting crop production. Chapter 4 on Pesticide degradation in soil is of particular interest not only to the agricultural scientists, but also to the environmental biologists. The factors affecting the persistence of pesticides in soil have been very well brought out, with supporting information. The table in pages 131-133 gives the common and chemical names of most of the pesticides in use in agriculture. Chapters 5 and 6 bring out the latest available information on humus and the interactions between humus and enzymes and other natural analogs including proteins and amino acids. The Chapter on Humus Biochemistry is dealt with in an excellent manner by three leading specialists from Germany, U.S.A. and Czechoslovakia.

The two volumes of Soil Biochemistry coming in succession after the first two volumes were published, are valuable additions to all libraries dealing with advanced biochemistry, microbiology and biology in general. While the first two volumes dealt with selected topics of general interest and knowledge, the Volumes 3 and 4 have covered newer areas which are complex and difficult to deal with. Together the four volumes of Soil Biochemistry would become the 'Bible' for biochemists dealing

with soil. The volumes should become very important reference books in every Science Library and Biochemistry Laboratory.

G. RANGASWAMI.

**Collected Papers of Sir Harold Jeffreys on Geophysics and Other Science.** Vol. 2—*Observational Seismology* (Gordon and Breach, London W. C. 2), pp. xxi + 697, Price £ 20.10.

The Volume on "Observational Seismology" is one of the six volumes of the collected scientific papers (excluding his books and the Jeffreys-Bullen Seismological Tables) of the veteran Mathematician-Geophysicist Sir Harold Jeffreys.

The Volume contains 40 papers of Seismology published by the author between the years 1935 and 1968 in various international journals of which 21 papers appeared (1935-54) in the *Geophysical Supplement of the Monthly Notices of the Royal Astronomical Society (MNGS)*, 11 in the *Geophysical Journal of the Royal Astronomical Society (RAS 1958-68)* and the remaining eight in other journals.

The subjects dealt with in these papers include:

- (1) Critical studies of the travel times of different types of earthquake waves as shown by the different phases in the seismograms obtained at different distances from the epicentre. These include both shallow focus and deep-focus earthquakes and earthquakes originating in different regions of the world.
- (2) Comparison of the accuracy of recording in the seismograms of seismological stations all over the world.
- (3) Critical and continued appraisal of the accuracy of the values of travel times as given in the Jeffreys-Bullen I.S.S. Tables of 1948 and tables by other authors.
- (4) After-shocks and periodicity of earthquakes.
- (5) Inferences regarding the structure of the interior of the earth as seen from the variation of travel times  $t$  of different phases at different angular distances ( $m$ ) from the epicentre of the earthquake to the seismic station.
- (6) Special discussion of travel times up to  $20^\circ$  and  $30^\circ$  and of Core waves.
- (7) Travel times of shock waves from two explosions, one in U.K. (1947) and the others in the Pacific (1962).

Institutions and research workers dealing with Seismology and the Physics of the Interior of the Earth will be well advised to obtain a copy of this Volume of papers by one of the founders of Modern Seismology.

K. R. RAMANATHAN.

**Dictionary of Data Processing.** By Maynard (Jeff). (Newnes, Butterworths, London), 1975. Pp. 269. Price £ 3.90.

Since the first appearance of a Dictionary in the field of Data Processing, the number of terms in the subject is ever increasing. This may be largely due to the fact that the advent of each new computer generation carries with it many new terms describing components, functions or procedures. The present book attempts to define terms in common practice in Data Processing Industry.

The Dictionary is designed for use primarily by computer users, computer and data process managers and others where work brings into contact with some aspects of data processing industry. It is alphabetically arranged covering more than 4000 terms and reflects modern developments in data processing industry.

So far as can be checked for a book of this size, the definitions appear technically accurate and they are crisp and informative. Where more than one term covers an entry, there is a cross reference. The shortest definitions run to one line and longest to a few lines occasionally with diagrams or tables.

The book comprises six appendices which include<sup>s</sup> list of common acronyms and abbreviations, standards relating to Data Processing, etc., and this is very useful to all interested in the industry.

The work is an important contribution to the development of a dictionary in the field of data processing. The printing and get-up of the volume are consistent with the high standard of performance that we have come to expect of publishers. The high cost would prevent individual researcher from owning a copy for ready reference. He will probably borrow his copy from the local library which ought to have one.

T. K. S. IYENGAR.  
G. S. R. RAO.

**Statistical Mechanics and Properties of Matter (Theory and Applications).** By E. S. R. Gopal. (Ellis Horwood Limited, Chichester; Halsted Press, a Division of John Wiley and Sons, N.Y. 10016, 1974). Pp. xvi + 302, Price \$ 27.50.

This is an excellent book coming out from the courses the author has given for many years to undergraduate students from various disciplines, such as applied mathematics, physics, engineering, etc., and

hence it reflects a general outlook rather than a specialized one.

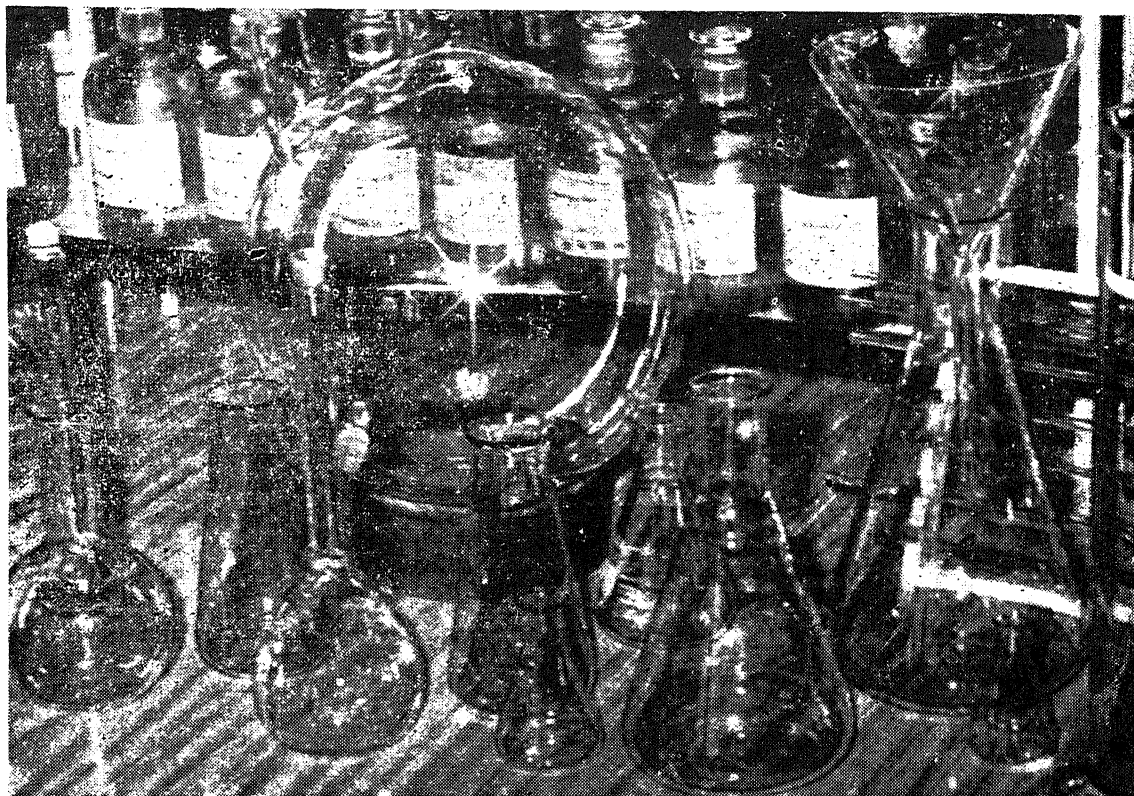
The author himself being a solid state physicist has some bias towards that branch in the treatment of physical aspects of matter. The book consists of three parts. Part A deals with Equilibrium Statistical Mechanics wherein he has derived classical and quantum distribution functions mainly from the partition functions. Part B deals with nonequilibrium statistical mechanics and he deals with Fluctuations, Onsager Relations, Kubo Relations, etc., Part C is devoted to applications of statistical methods to physical problems. In this, theory of thermionic emissions, photoelectric effect, electrons in metals, phase transition are all discussed. From this point of view this book is a very useful one for an undergraduate or even a graduate student. The large number of problems at the end of each Chapter and their solutions at the end of the book is a feature which one misses in many books on this subject.

There are some obvious printing mistakes also in the book. Right on the first page equation (1) is wrongly printed. Also there are some usually common mistakes in such books here as well. Thus, at the end of the page 19, the author justifies the maximisation of  $\ln W$  instead of  $W$  because that happens to be easier to handle: one takes an extremum of  $\ln W$  because it is  $\ln W$  (and not  $W$ ) which is an additive invariant and hence can be expressed as a linear function of the other additive invariants such as linear momentum, angular momentum, number density, energy, etc., as pointed out by Poincare. Also the distinguishability in classical statistics as against indistinguishability in quantum statistics is not brought out clearly, for equation (1.12) on page 19 is invariant under permutation of particles and hence also implies indistinguishability.

Again the very active and growing domain of nonequilibrium statistical physics has only been very casually treated. Of course, that could not have been included in a volume of this size, but a book in which equilibrium statistical mechanics comes as an asymptotic limit (in time) of a non-equilibrium theory would have been a more logical way of thinking. Nevertheless this book could be recommended without any reservation to a serious student of statistical physics because of its growing importance. The price of this book (\$ 27.50) is a bit too high.

R. PRATAP.





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## SHRI HARI OM ASHRAM VIKRAM SARABHAI RESEARCH AWARDS

Four awards are to be made to Indian Scientists, who are not above 45 years of age on 1st January 1975, for original work in the following fields :

- (1) Electronics and Telecommunications ;
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- (4) Systems Analysis and Management Problems.

The last date for receiving nominations will be 31st December, 1975. Sponsors are requested to send a note summarising the contributions, achievements and list of publications of the sponsored candidate together with his/her bio-data.

D. LAL  
*Director,*  
PHYSICAL RESEARCH LABORATORY,  
NAVRANGPURA,  
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# GRAVITY SURVEY AND QUALITATIVE APPRAISAL IN A PART OF LOWER GODAVARI VALLEY, A.P.

V. BHASKARA RAO\* AND P. D. VENKATESWARLU\*\*

## ABSTRACT

A Bouguer gravity map was prepared occupying about 700 gravity stations in a limited area of about 9,000 sq. km in a part of the Lower Godavari Valley of Andhra Pradesh. The established auxiliary base gravity values and the measured density values of various formations in the surveyed area are given.

The Bouguer anomaly trends obtained are highly regular when correlated with the surface geological formations. A qualitative appraisal of these anomalies brought out some interesting features supporting the possible extension of the Gondwanas below the alluvial cover and showing the presence of some basement ridges.

## INTRODUCTION

THE present work forms a part of the regional gravity investigations carried out by the Andhra University, under a research scheme sponsored by the CSIR, New Delhi, in a part of the Lower Godavari Valley lying in the West Godavari and Krishna Districts of Andhra Pradesh. No systematic geophysical survey was carried out in the area, till the regional magnetic work undertaken by Krishnabrahmam (1962) and Sitapatirao (1963) in a part of the present surveyed area. Compiling all the available gravity data, namely, those of Survey of India and ONG Commission with the NGRI occupied stations, Queresby *et. al.* (1968) presented a regional Bouguer gravity map for the entire Gondwana basin right from Sironcha in the north-west up to the coast in the south. The present gravity surveys were conducted in a limited part of the Lower Godavari valley establishing about 700 gravity stations in an area of about 900 sq. km. In fact, the observed Bouguer gravity anomalies when correlated with the surface geology revealed some interesting and useful information.

## GEOLOGY

In the Godavari Valley, the Gondwana rocks are extensively developed from the Chandrapur District (Maharashtra State) in the north, down to the West Godavari District in Andhra Pradesh (the present area of investigation). Detailed geological mapping was carried out by Blandford (1871) and King (1872-82). Later, working with Geological Survey of India, Krishnan (1960) has contributed valuable information regarding these coal fields. Detailed geological surveys on Gondwanas were carried out by a number of workers, viz., Venkayya (1947), Apparao (1952), Ramachandra Raju (1952), Sarma (1957) and Sathiraju (1959) among others of

Andhra University. A generalised regional geological map of the area under investigation compiled from the results of the above is shown in Fig. 1.

| Formation                         | Equivalent                                     | Age               |
|-----------------------------------|--|-------------------|
| Godavari Alluvium                 | Alluvium                                       | Recent            |
| Rajahmundry Sandstones            | Cuddalore Sandstones                           | Miocene           |
| Godavari traps and intertrappeans | Linga and Chindwara flows and inter-trappeans. | Cretaceous-Eocene |
| Infra-trappeans                   | Lametas of Jabalpur                            | Upper Cretaceous  |
| Tirupati Sandstones               | Chikiala                                       | Upper Jurassic    |
| Raghavapuram Shales               | Kota   | Upper Jurassic    |
| Gollapalle Sandstones             | Kamthis  | Lower Triassic    |
| Barakar Sandstones                | Barakar  | Lower Permian     |
| Kondalite Series                  | Charnockites<br>Khondalites<br>Gneisses        | Archeans          |
| Unclassified Crystallines         | Peninsular Gneisses                            |                   |

## PLAN AND PROCEDURE OF THE SURVEY

The present investigations cover an area of about 9,000 sq. km. bounded by north latitudes 16° 30' to 17° 15' and the east longitudes 80° 45' to 81° 45' and was covered by 12 (one inch to a mile) Survey of India toposheets. A total of 700 gravity stations with a density of 1 station per 13 sq. km. were set up in the area (Fig. 2). All the gravity values are connected to the Survey of India station at Vijayawada (16° 30' 19", 80° 37' 46") with a value of 978.4515 (Gulatte, 1956) which is the primary base

\* Andhra University, Waltair.

\*\* Geological Survey of India, Central Region, Nagpur.

FIG.1-GEOLOGICAL MAP OF A PART OF GODAVARI VALLEY A.P.

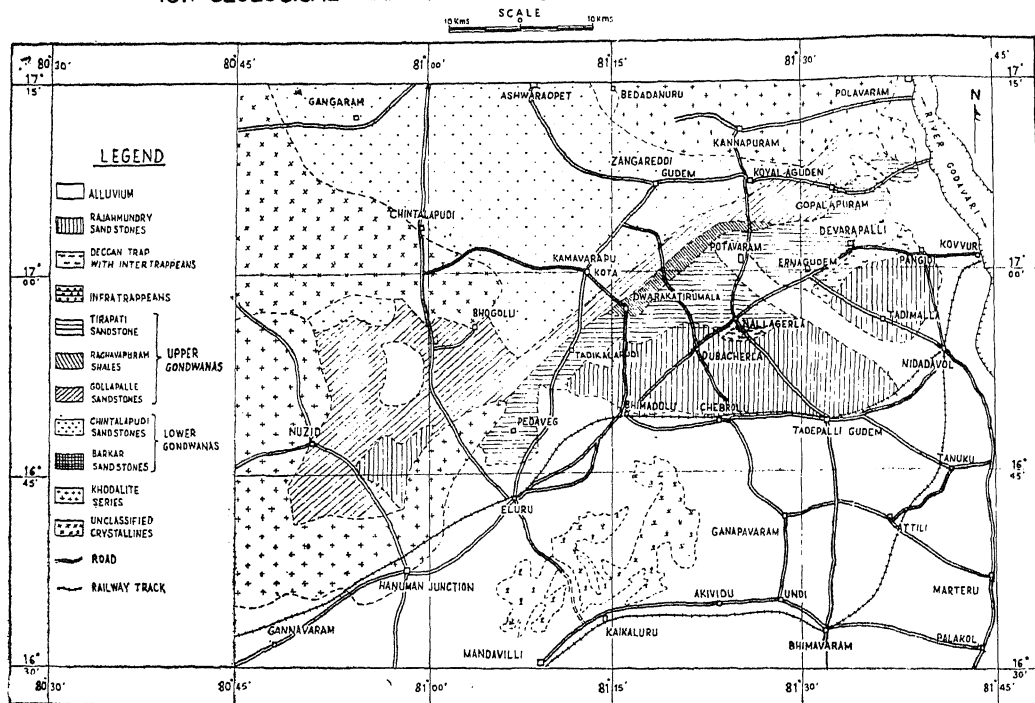
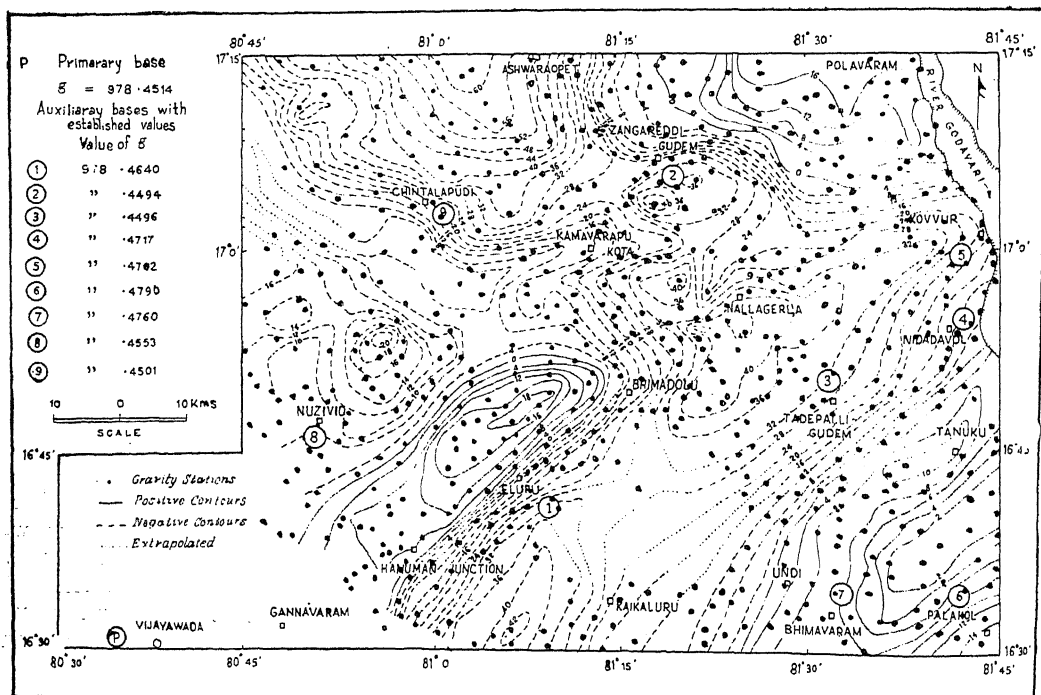


FIG.2.- BOUGUER GRAVITY MAP IN A PART OF  
LOWER GODAVARI VALLEY A.P.



for the entire survey. Applying the usual corrections assuming a density of 2.67 gm/cc, the reduced Bouguer gravity to the mean sea level is shown in Fig. 2, along with the established auxiliary bases and the station locations. Over forty rock samples were collected from the different geological formations at various places. Care has been taken to obtain fresh, unweathered samples and in many places samples were collected from quarries and other wells. In measuring density for porous rocks, namely, the sedimentary rocks, the method given by Garland (1965) is adopted. The average of measured values of density for each formation are shown in Table I.

TABLE I

| Sl. No. | Formation                 | No. of Samples | Average density value gm/cc |
|---------|---------------------------|----------------|-----------------------------|
| 1.      | Rajahmundry Sandstone     | 5              | 2.18                        |
| 2.      | Deccan Traps              | 6              | 2.82                        |
| 3.      | Upper Gondwanas:          |                |                             |
|         | Tirupati Sandstones       | 4              | 2.02                        |
|         | Raghavapuram Shales       | 3              | 2.97                        |
|         | Gollapalli Sandstones     | 4              | 2.08                        |
| 4.      | Lower Gondwanas:          |                |                             |
|         | Chintalapudi Sandstones   | 5              | 2.10                        |
|         | Barakar Sandstones        | 3              | 2.11                        |
| 5.      | Khondalite Series:        |                |                             |
|         | Charnockites              | 4              | 2.72                        |
|         | Khondalites               | 3              | 2.57                        |
| 6.      | Unclassified Crystallines | 5              | 2.60                        |

#### DISCUSSION OF THE BOUGUER ANOMALY MAP

The Bouguer anomaly reflects the composite effect of many factors namely intra-crustal structures, intrusions which may or may not reach the surface and surface geological inhomogeneities. The Bouguer anomalies obtained, when correlated with the surface geology, reveal the following factors. The trend and pattern of anomalies associated with the Lower Gondwana sediments are highly regular. The Lower Gondwanas, namely, the Chintalapudi sandstones, show a maximum negative anomaly of 62 mgal west of Ashwaraopet. The magnitude of this anomaly gradually decreases to the south with a - 28 mgal anomaly contour practically coinciding with the boundary of this formation. The trend of the negative anomalies, lying between 8 and 28 mgals, which is east to west in this region, takes a turn between Kamavarapukata and Zangareddigudem and follows the strike of the

Upper Gondwana formations. The closure near Zangareddigudem of about 40 mgals negative anomaly probably indicates thickening of the sediments in this area. The magnitude of the anomalies, in general, indicates less thickness for the Upper Gondwana formations than the Lower Gondwanas.

The remarkable feature in the Bouguer anomaly map is a band of parallel contours trending north-west-southeast direction, from Ashwaraopet to Kovvur with an anomaly variation of 30 mgals. This anomaly pattern, which closely follows the sedimentary boundary, might represent the sloping of the crystallines towards the sediments. Similar pattern of contours was observed from Kovvur, Tadepallegudem down to Kaikaluru in the far south, on the area covered by tertiaries and recent alluvium formations. A similar approach can be made for understanding this anomaly pattern from Kovvur Tadepallegudem to Kaikaluru. Thus, it may be envisaged that the above pattern of contours running through Ashwaraopet-Kovvur-Tadepallegudem and Kaikaluru limits the eastern margin of the sedimentary basin. In the western side, the anomaly contours from Tadikalapudi, Kamavarapukata and Chintalapudi roughly follow the sedimentary crystalline boundary.

The closure at Nallagerla and just west of Nallagerla are probably due to the thick sedimentary columns, as in the case of the closure at Zangareddigudem. The Deccan trap formations near Pangidi and Nallagerla do not show up in the Bouguer anomaly map probably due to their very limited areal extension and thickness. A closure of about - 20 mgals is observed north of Nuzvid. This may be due to the limited exposed patch of lower Gondwanas just outside the basin.

Regarding the anomalies outside the area of the basin, the noteworthy one is the strikingly elongated positive closure of the order of 18 mgals extending in a southwest-northeast direction from Tadikalapudi. This anomaly starts from Gannavaram and extends upto Tadikalapudi in the above strike direction. From the surface geology, metamorphics were outcropping near Gannavaram and extending in the same strike direction of southwest-northeast, upto Pedavegi, with Upper Gondwanas on the northern side and recent alluvium on the southern side. These elongated positive anomalies may thus be correlated with the metamorphic structure, even in its subsurface portions.

The positive anomalies in the eastern part of the area Polavaram and the positive anomalies in the region of Bhimavaram-Tanuku with a 10 mgal closure near Tanuku need some elucidation. The positive anomalies in the vicinity of Polavaram may be due to variations of density in the

Khondalite formations produced possibly by intrusions of Charnockites, particularly near Prakkilanka ( $17^{\circ} 8' 0''$ ;  $81^{\circ} 40' 30''$ ) as discussed by Sitapati-rao (1963) and as evidenced from outcrops north of Polavaram. However, the gravity effect of these intrusions may be quantitatively inadequate to account for the observed Bouguer anomalies. It may also be pointed out that the southern limit of this positive anomalies roughly coincides with the Archaean basement boundary. Also these positive anomalies are known (Queresby *et al.*, 1968) in the eastern ghats strike direction, i.e., NE-SW. This may point to the possibility of a structure faulting on the eastern edges of the basin and parallel to the general strike direction of the eastern ghats. It is, however, not possible to establish this aspect unless more detailed investigations are undertaken.

The elongated positive anomaly closure near Tanuku and Bhimavaram is quite interesting particularly as it is situated over an area covered by tertiaries and alluvium. Taking a maximum thickness of about 1,000 feet for tertiaries (Balasundaram, 1969) and of about 500 feet for alluvium, there is hardly room for a structure large enough to produce this 12 mgal anomaly. Thus the observed anomaly has to be visualised as arising due to the effect of something within the basement. With this background, it is very difficult to distinguish an anomaly which might be a result of moderate folding or faulting in the sediments, or of moderate relief of the basement surface. Bhaskara Rao and Satyanarayana Murty (1969) from their magnetic studies in parts of the alluvial areas of the East Godavari District, just adjacent to the present area (on the other side of Godavari river) pointed the possible presence of a ridge-like basement structure running ENE and plunging in the WSW direction. During their extensive geophysical surveys for oil structures, the ONG Commission reported the same order of anomaly at Tanuku and attributed the same, primarily in the variation in basement topography (Ramana, 1961-62; Das *et al.*, 1970). Also these two basement ridges discussed above have been demarcated by the ONG Commission in their Tectonic Map of India (1968) based on the geophysical results. The observed gravity anomalies are in alignment with the strike of the reported ridge. Thus, this anomaly may be due to ridge-like feature of sufficient relief in the basement. It is, however, interesting that, a high negative parallel band of contours from Nallagerla to Kaikaluru with positive anomalies on either side, all with the same general strike direction, are observed in the southern parts of the area surveyed. This negative band of contours can only be explained accounting the presence

of Gondwanas below the alluvial cover. In fact, the basement structure derived from the gravity results, assuming a reasonable thickness of alluvial cover in these parts is very informative and is being presented separately with tectonic history of the Lower Godavari Valley (Bhaskara Rao and Venkateswarlu, under preparation).

#### CONCLUSIONS

A systematic gravity survey was conducted in a part of the Lower Godavari Valley of Andhra Pradesh. A network of 9 auxiliary bases were established during the course of the survey. Densities of the surface rocks were determined in the laboratory taking representative samples from all the geological formations in the area.

The trend of the Bouguer anomalies is highly regular demarcating the various geological formation boundaries. The Lower Gondwanas are characterised by a "Low" negative anomaly of 62 mgals west of Ashwaraopet. It is suggested that the band of contours with an anomaly variation of about 30 mgals running through Ashwaraopet Kovvur-Tadepallegudem and Kaikaluru limits the eastern margin of the sedimentary formations. The present gravity survey supports the earlier views about the existence of the subsurface ridges at Tanuku and north of Eluru and delineated their extensions. Also, the run of negative contours from Kovvur-Tadepallegudem-Kaikaluru, flanked by positive elongated contour closures, suggests the presence of Gondwana sediments below the alluvial cover in these parts of the area.

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# EFFECT OF 2, 4-DINITROPHENOL AND ATP ON UPTAKE, TRANSLOCATION AND DISTRIBUTION OF $^{32}\text{P}$ IN COTTON PLANTS UNDER DIFFERENT LIGHT CONDITIONS

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## ABSTRACT

Cotton seedlings were subjected, in light and darkness, to DNP, ATP or both for 3 hours, then allowed to remain in contact with  $^{32}\text{P}$  for further 6 hours. Darkness decreased phosphorus uptake and its translocation. In the light, DNP decreased the uptake of  $^{32}\text{P}$ , but enhanced its translocation to the shoot. ATP did not affect  $^{32}\text{P}$  uptake but enhanced its translocation. ATP could not overcome the negative effect of DNP on  $^{32}\text{P}$  uptake. In the dark, neither DNP nor ATP exerted significant effect on  $^{32}\text{P}$  uptake, yet accelerated its translocation. Under both light conditions, DNP accumulated most of the translocated  $^{32}\text{P}$  in the stem, while ATP moved it further to the leaves. The enhancement of  $^{32}\text{P}$  translocation was accompanied by the presence of higher percentage of  $^{32}\text{P}$  in an organic form. It was concluded that high energy compound formed during photosynthesis may play a role in the metabolic active uptake of phosphorus.

## INTRODUCTION

THE uptake of phosphorus and its translocation in plants were suggested to be metabolic active processes (Brower, 1965 and El-Fouly and Ashour, 1969). The energy required for such a process is supplied from the adenosine-triphosphate (ATP) deposited in the cells (Weigh, 1963). The high energy compound (ATP) is produced during the process of oxidative phosphorylation (Jackson *et al.*, 1962). Thus, 2,4-dinitrophenol (DNP), an inhibitor of oxidative phosphorylation was found to decrease the uptake of phosphorus by plant roots (Stenlid, 1959).

The uptake of phosphorus by roots and its translocation to the shoot are higher in the light than in the dark (Linser, 1965), and increase with increasing light intensity (Ashour *et al.*, 1968). Raven (1969) suggested that the ATP required for regulation of ion pump can be produced in the light by cyclic photosynthetic phosphorylation.

The aim of this work was to investigate the effect of DNP and ATP on  $^{32}\text{P}$  uptake, translocation and distribution in the cotton seedlings in light and in darkness.

## MATERIALS AND METHODS

One month old, uniform cotton seedlings (*G. baradense*) cv. Ashmouni grown in water culture were selected. The plants were rinsed in large test tubes (2.5 cm dia and 20 cm long) filled with distilled water and left over-night. On the second day, the distilled water in each test tube was replaced by 50 ml of 1/4 strength complete Hoagland's nutrient solution containing  $10^{-4}$  M 2,4-dinitrophenol (DNP),  $10^{-3}$  M ATP (the dipotassium salt of ATP) or both and left for 3 hours. Then  $8\mu\text{Ci}$  of  $^{32}\text{P}$  as  $\text{KH}_2\text{PO}_4$  supplied from the Egyptian Atomic Energy Establishment was injected in the nutrient solution in each tube, and the plants were allowed to remain in contact with the  $^{32}\text{P}$  for 6 hours at  $21^\circ\text{C}$ . This experiment was conducted under conditions of both light (20,000 Lux) of fluorescent lamps, and darkness. Each treatment under both conditions had seven replicated tubes, thus each treatment included seven plants. At the end of the incubation period, the plants of four replicates were harvested, and the roots were washed carefully with running water for 2 min, then the plants were divided into roots,



stems and leaves and oven-dried separately at 105° C. Samples from different parts were prepared for counting on the same day. The shoots of the other three plants from each treatment were cut off 1 cm above the base. Filter paper (2 cm dia) was placed on the stems section for 15 min. to absorb all the translocated  $^{32}\text{P}$  towards the stem of the plant. The radioactive circles were dried, counted, then washed with successive portions of 7% and 2% ice-cold trichloroacetic acid (TCA)—and finally with water to discard the inorganic phosphorus (Tobbert and Wiebe, 1955). The TCA-treated circles were counted once more. The percentage of inorganic- and organic- $^{32}\text{P}$  was calculated.

### RESULTS

Under light conditions, DNP significantly decreased the uptake of  $^{32}\text{P}$ , whereas ATP was without significant effect (Table I). The addition of ATP together with DNP did not change the uptake of  $^{32}\text{P}$  more than that induced by DNP alone. In darkness, cotton plants absorbed much less amount of  $^{32}\text{P}$  than in light. Under dark conditions, neither DNP nor ATP had any significant effect on the uptake of  $^{32}\text{P}$  by plant roots as compared with the control plants. Combined treatment with DNP + ATP in the dark did not affect the uptake of  $^{32}\text{P}$ .

Table I also shows that the darkness markedly retarded the translocation of absorbed  $^{32}\text{P}$  from the root to the shoot of cotton plants. All treatments of metabolically active substances enhanced the translocation of the absorbed  $^{32}\text{P}$  from the root to the shoot under both light conditions, the combined treatment of DNP + ATP was the most effective one.

In Table II it is clear that in the light DNP and ATP retained more or less an equal percentage of  $^{32}\text{P}$  in the roots. However, much more of the  $^{32}\text{P}$  that moved out of the roots in DNP-treated plants remained in the stems in comparison with the ATP-treated plants, where, further moving of  $^{32}\text{P}$  towards the leaves was observed. In the dark, most of the absorbed  $^{32}\text{P}$  was retained in the roots, while only traces were found in the stem and the leaves as compared with that in the light. However, the change in  $^{32}\text{P}$  distribution among different plant organs in the dark due to DNP or ATP treatments was the same as observed in the light. When DNP and ATP were applied together most of the translocated  $^{32}\text{P}$  under light conditions was accumulated in the leaves; whereas in darkness it was retained mainly in the stem.

Table III shows that in the light, all treatments increased the percentage of organic- $^{32}\text{P}$  in the root

TABLE I  
Effect of DNP and ATP on the uptake and the transport of  $^{32}\text{P}$  in cotton plants in light and in darkness

| Treatment | The uptake of $^{32}\text{P}$ |      |                        |      |                  |      |
|-----------|-------------------------------|------|------------------------|------|------------------|------|
|           | $\times 10^3$                 |      | $\times 10^3$          |      | Transport index* |      |
|           | counts/sec/<br>plant          |      | counts/sec/<br>g. root |      |                  |      |
|           | Light                         | Dark | Light                  | Dark | Light            | Dark |
| Control   | 326                           | 213  | 1270                   | 620  | 32.1             | 13.1 |
| DNP       | 275                           | 201  | 631                    | 732  | 64.3             | 32.2 |
| ATP       | 306                           | 180  | 1062                   | 599  | 65.4             | 28.5 |
| DNP+ATP   | 237                           | 208  | 787                    | 702  | 86.6             | 68.6 |
| P=0.05    | 37                            |      | 123                    |      |                  |      |

$$\text{*Transport index} = \frac{^{32}\text{P in shoot}}{^{32}\text{P in whole plant}} \times 100$$

TABLE II  
Effect of DNP and ATP on the distribution of  $^{32}\text{P}$  in cotton plant in light and in darkness

| Treatment | Roots                             |      | Stem  |      | Leaves |      |
|-----------|-----------------------------------|------|-------|------|--------|------|
|           | Light                             | Dark | Light | Dark | Light  | Dark |
|           | $\times 10^3$ counts/30 sec/organ |      |       |      |        |      |
| Control   | 221                               | 185  | 92    | 26   | 12.3   | 1.5  |
| DNP       | 98                                | 137  | 163   | 64   | 13.8   | 0.8  |
| ATP       | 106                               | 129  | 117   | 46   | 83.2   | 5.4  |
| DNP+ATP   | 32                                | 65   | 84    | 100  | 121.6  | 42.3 |
| P=0.05    | 28                                |      | 18    |      | 2.8    |      |
|           | % of total radioactivity          |      |       |      |        |      |
| Control   | 67.9                              | 86.9 | 28.3  | 12.4 | 3.8    | 0.7  |
| DNP       | 35.7                              | 67.8 | 59.3  | 31.8 | 5.0    | 0.4  |
| ATP       | 34.6                              | 71.5 | 38.2  | 25.5 | 27.2   | 3.0  |
| DNP+ATP   | 13.4                              | 31.4 | 35.4  | 48.2 | 51.2   | 20.4 |

TABLE III  
Effect of DNP and ATP on the percentage of organic and inorganic  $^{32}\text{P}$  in the root exudate of cotton plant in light and in darkness

| Treatment | Organic- $^{32}\text{P}$ |      | Inorganic- $^{32}\text{P}$ |      |
|-----------|--------------------------|------|----------------------------|------|
|           | Light                    | Dark | Light                      | Dark |
| Control   | 49.7                     | 68.6 | 50.3                       | 31.4 |
| DNP       | 69.2                     | 84.3 | 30.8                       | 15.7 |
| ATP       | 79.3                     | 66.0 | 20.7                       | 34.0 |
| DNP+ATP   | 79.6                     | 91.3 | 20.4                       | 8.7  |

exudate at the expense of the inorganic fraction. Darkness showed similar effect. Under such dark conditions, DNP also increased the percentage of organic- $^{32}\text{P}$  in the root exudate, ATP was ineffective, while DNP + ATP appreciably increased it as compared with that in the control plants.

#### DISCUSSION

The results indicate that light enhances the uptake of phosphorus by the roots of cotton plants and its translocation towards the stem thus confirming the results obtained by others (Linsner, 1965 and Ashour *et al.*, 1968). Such effect was suggested by McEvoy (1967) to be due to the increased supply of the photosynthate under light conditions. In the light, the decrease in the uptake of  $^{32}\text{P}$  after treatment with DNP, may be due to the inactivation of the phosphorylation processes in plant tissues. Under such conditions the formation of ATP was found to be partially blocked (Jackson *et al.*, 1962). However, in the dark, when only the oxidative phosphorylation was acting and not the photosynthetic phosphorylation, the DNP and the ATP were without effect on the uptake of  $^{32}\text{P}$ . Thus, it seems that the high energy compounds formed during photosynthesis alongside with the downwards photosynthate may take part in the metabolic active uptake of phosphorus. On the other hand, when ATP is present in the root medium, the uptake of  $^{32}\text{P}$  was not activated, but on the contrary, may be slightly retarded. A competitive effect between the molecule of ATP or its derivatives and the ion of phosphorus for a certain carrier was suggested for the explanation of such phenomenon (Vakhmistrov and Listova, 1967).

The translocation of  $^{32}\text{P}$  from the root to the shoot was enhanced under both conditions of light due to DNP or ATP treatments, while DNP + ATP seemed to have an additive effect. It seems that high energy phosphorus compound is required for translocation. Randall and Vose (1963) found that DNP had a major positive effect on the translocation of phosphorus to the shoots. In addition, it seems that when the translocation of phosphorus from the root was enhanced, the organic fraction of the translocated phosphorus was increased indicating a change in phosphorus metabolism. Further studies are needed to clarify the problem of translocation of phosphorus compounds in connection with the role of DNP, ATP and light.

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### PROPAGATION OF *DIOSCOREA FLORIBUNDA* FROM IN VITRO CULTURE OF SINGLE-NODE STEM SEGMENTS

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#### ABSTRACT

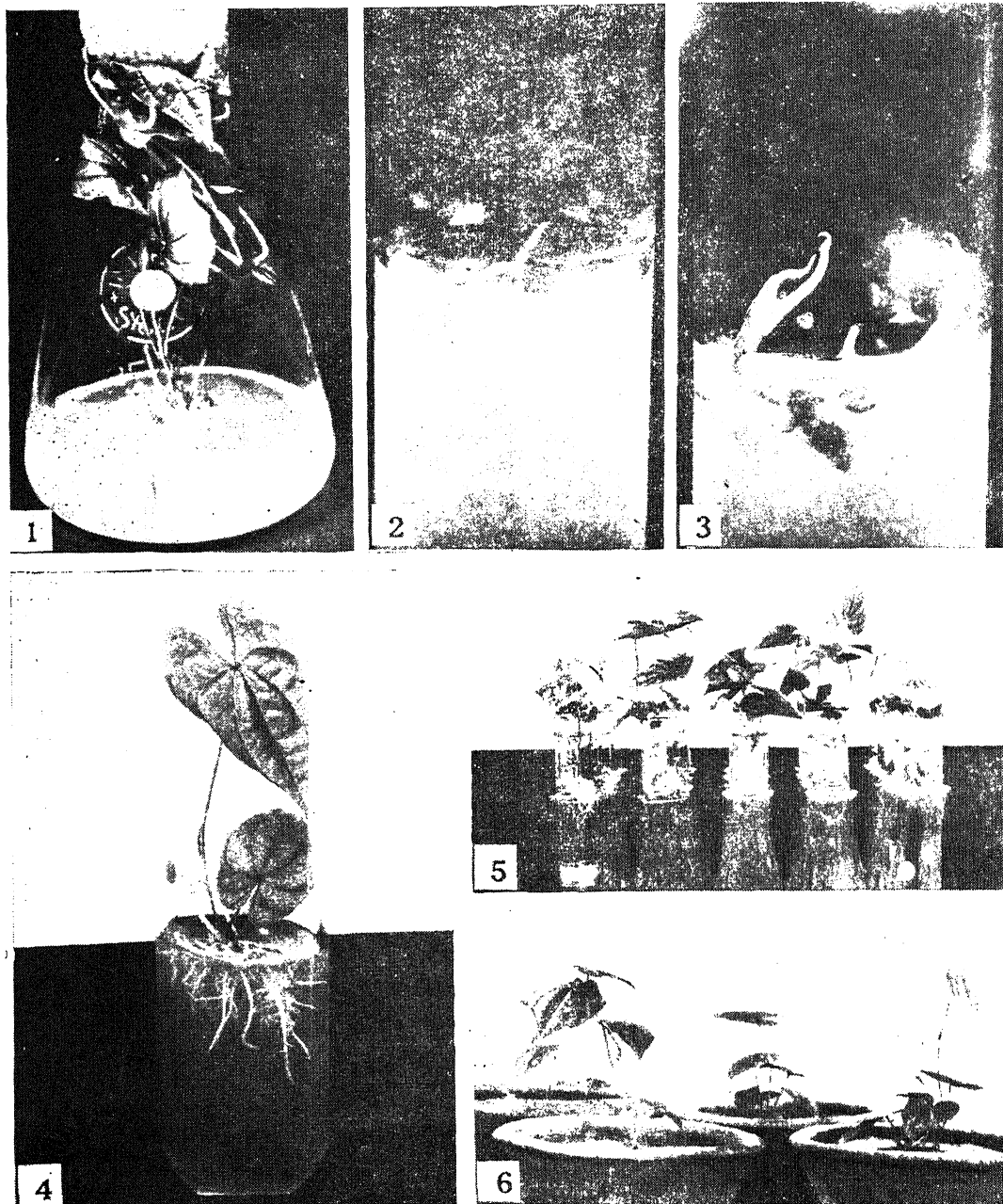
*Dioscorea floribunda* plants were established in aseptic cultures from surface-sterilized single-node stem segments of field-grown vines. Axillary buds of nodal segments proliferated in presence of 6-benzylaminopurine (2 mg/l) unaccompanied by root formation. Whereas, shoot apices and single-node leaf cuttings rooted 100% in presence of NAA (0.5 mg/l), resulting into plantless, 100% of which were successfully grown in potted soil. It took about 40 days to obtain a 5-6 leaved plantlet in potted soil from single-node cutting taken from a plant grown in aseptic culture.

#### INTRODUCTION

*DIOSCOREA FLORIBUNDA* Mart. and Gal. is one of the three *Dioscorea* spp. (the other two are *D. composita* Hemsl. and *D. deltoidea* Wall.)

commercially yielding diosgenin, a main precursor, from plant source, for the synthesis of steroidal drugs, namely, cortisone, sex hormones, oral contraceptive pill, etc., which are so important in

modern medicine. The great medicinal value of *Dioscorea* necessitates its large-scale cultivation as a crop since wild resources can hardly meet the ever-increasing demand for diosgenin. To avoid



FIGS. 1-6. Cultures of *Dioscorea floribunda*. Fig. 1. A plant in aseptic culture obtained from single-node stem segment of field-grown vine ( $\times 0.7$ ). Fig. 2. A single-node stem segment taken from an aseptically growing plant as it looked at the time of inoculation ( $\times 1.71$ ). Fig. 3. Proliferation of axillary bud of an explant as shown in Fig. 2 ( $\times 1.8$ ). Fig. 4. A rooted shoot ( $\times 0.9$ ). Fig. 5. Liquid culture of rooted shoots—plantlets—in an inorganic nutrient solution ( $\times 0.27$ ). Fig. 6. About 50-day-old plantlets, regenerated from single-node cutting, growing in potted soil ( $\times 0.3$ ).

variation, its high yielding strains have to be multiplied vegetatively. Propagation from tuber segments is a slow process, whereas consistent success for its rapid propagation from leaf or stem cuttings has not been achieved and with certain commercial species the latter method has failed<sup>1</sup>. *D. floribunda* could be propagated from single-node leaf cuttings<sup>2,3</sup>, but as reported by Bammi and Randhawa<sup>4</sup> the percentage of rooting of such cuttings has been quite low, i.e., only 20–30%. Application of the methods of tissue and organ culture for induction and proliferation of shoot buds and their 100% rooting, as has been demonstrated for *Nicotiana*<sup>5</sup>, *Chrysanthemum*<sup>6</sup> and *Citrus*<sup>7</sup>, holds a great promise for rapid multiplication of *Dioscorea* too. Preliminary results of such a study for propagation of *D. floribunda* are reported here.

#### EXPERIMENTAL PROCEDURE

About 2-cm-long stem segments consisting of a single node, a portion of petiole with its axillary bud and small portions of internode on either side were obtained from middle region of vigorously growing stem of *D. floribunda* vines cultivated at the National Botanic Gardens, Lucknow. The segments were pretreated with a detergent (Teepol 5%) for 5 min. and surface-sterilized by first dipping them in 95% ethanol for 5 sec. followed by immersion in HgCl<sub>2</sub> solution (0.2%) for 30 min. Such segments, after being thoroughly washed with sterile distilled water, were inoculated one per culture tube with their cut basal ends inserted in the nutrient agar.

A modification of Murashige and Skoog's medium<sup>8</sup> was used as the basal medium. The medium was adjusted to pH 5.8 and sterilized by autoclaving at 1.08 kg/cm<sup>2</sup> for 15 min. The cultures were grown under 3000 lux fluorescent light for 14 hr. daily at 27° ± 1° C. Humidity of the culture room was maintained at 70 ± 4%.

#### RESULTS

Stem segments cultured in the basal medium supplemented with 15 mg/l adenine sulphate and 0.1 mg/l NAA remained quiescent for about 20 days, after which swelling appeared at the site of axillary bud denoting the formation of new tuberous tissue, from which roots came out first followed by the development of one or two shoots. Such plantlets, on being subcultured in the same medium contained in Erlenmeyer flasks, grew vigorously giving rise to more shoots (Fig. 1), and constituted the source material for further cuttings.

Single-node explants (Fig. 2) taken from *in vitro*-growing plants when cultured in a medium containing 2 mg/l 6-benzylaminopurine produced

5–6 or sometimes more shoot buds from its axil after an incubation of 20–25 days (Fig. 3). In this way, multiplication of shoots was effected. Shoot apices (1–2 cm long) as well as single-node leaf cuttings from *in vitro*-growing plants 100% rooted profusely in a medium containing 0.5 mg/l NAA within a period of 10–15 days (Fig. 4). A large number of such cuttings could be obtained from a single culture of aseptically growing plant as several "crops" of new shoots continued to develop from the rooted basal portion of the plant after the excision of older shoots.

The rooted leafy shoots, after being reared for about 20 days on an auxin-free medium supplemented with 25 mg/l adenine sulphate to promote shoot growth, were then taken out from aseptic cultures and grown in a liquid inorganic nutrient medium (Fig. 5). When the plantlets got acclimatized during their liquid culture for 10–15 days, they were transplanted into sterilized potted soil (Fig. 6). For the first 2–5 days after transfer in liquid culture and in potted soil, the plantlets were covered by glass-jars to prevent them from desiccation. All the plants transplanted in this way grew normally and vigorously in potted soil.

It took about 40 days to obtain 5–6 leaved plantlets in potted soil from the cuttings taken from an aseptically growing plant. Further experimentation is continuing to achieve still faster rate of multiplication of plantlets before this process could be developed as a practical method for clonal propagation of *Dioscorea*.

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## STUDIES ON THE EFFECT OF AMINOTRIAZOLE ON CHLOROPLAST DEVELOPMENT IN *PHASEOLUS RADIATUS* L.

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### ABSTRACT

The effect of 3-amino-1, 2, 4-triazole (amitrole) on the development of chloroplast is studied by exposing dark grown seedlings of *Phaseolus radiatus* L. to light. The syntheses of chlorophyll and carotenoid pigments, as a function of chloroplast development, were found to be affected specifically. The inhibitory action of amitrole on carotenoid level is more pronounced than on chlorophyll content.

A few aminoacids, riboflavin and ethylene diamine tetra-acetate (EDTA) were found to effectively reverse the inhibitory effect of aminotriazole when administered concurrently. Likewise ferric and magnesium ions also nullified the toxic effect of this compound. A possible mode of action of this substance, in interfering with the development of chloroplast is discussed, in the light of the above observations.

### INTRODUCTION

THE herbicide 3-amino-1, 2, 4-triazole is widely used as a defoliant and as a growth inhibitor. This compound is found to produce albinism—a state in which chloroplast development is completely inhibited—in higher plants without any marked effect on the cellular metabolism or the morphology of the leaves at sub-lethal concentrations. The extensive work carried out to understand its mode of action has been reviewed<sup>1-2</sup>. Though its primary site and mode of action in heterotrophic micro-organisms is adequately explained, definite information with reference to its site and mechanism of action in higher plants—particularly a basis for its selective inhibition of the chloroplast development—seems yet to be understood. This paper reports our studies on the effect of this compound on the development of chloroplasts with special reference to the synthesis of chlorophyll and carotenoid pigments in the young leaves of *Phaseolus radiatus* L. seedlings under conditions of maximal chloroplast development.

### MATERIALS AND METHODS

#### Plant Material

*Phaseolus radiatus* L. seedlings used in this study were raised on sandy loam in plastic trays for 7 days either in the dark or in light from a bank of fluorescent lamps, at an intensity of 4,200 lux. Only primary leaves of the seedlings were used throughout, for analytical studies. In greening experiments, the dark grown seedlings were pretreated, before exposure to light, with 5 and 10 mM aminotriazole for 24 hours by keeping their roots immersed in the test solutions.

#### Reversal of Amitrole Bleaching

In experiments designed to study the reversibility of amitrole bleaching, the 6-day old dark grown

seedlings were treated in the dark for 24 hours with solutions of equimolar (5 mM) concentrations of amitrole plus aminoacids, or other substances like metal ions, EDTA and riboflavin. After pretreatment in the dark for 24 hours, the seedlings were exposed to light for an additional 24 hours in the same solutions.

#### Experiments with Excised Leaves

To study the effect of amitrole on greening, two sets of leaves from 7-day old dark grown seedlings were pretreated in petri dishes with 5 mM amitrole and distilled water separately by floating in dark for 24 hours before transferring to light. Leaf samples were removed at indicated time intervals and their pigments estimated.

#### Estimation of Chlorophyll and Carotenoids

The pigments were extracted from 100 mg leaf samples by grinding in a tissue homogenizer with 80% aqueous acetone. The chlorophyll and carotene contents in the acetone extract were estimated spectrophotometrically according to the methods of Arnon<sup>3</sup> and Goodwin<sup>4</sup> respectively.

### RESULTS

The effect of aminotriazole on the development of chloroplast in the leaves of dark grown *Phaseolus* seedlings on exposure to light is shown in Table I. Normally, the chloroplasts in *Phaseolus* seedlings take about 20 hours for the complete development as measured in terms of chlorophyll content. When fully developed, they contain about 2 mg of chlorophyll per gram fresh weight of the leaf. The chloroplast development was retarded by 25 and 50% respectively in 5 and 10 mM amitrole-treated seedlings. At concentrations higher than 10 mM, amitrole seemed to act as a non-specific toxin affecting root growth, leaf size, etc., and hence in

TABLE I

Effect of amitrole on the development of chlorophyll and carotenoid in dark grown seedlings of *Phaseolus* on exposure to light

| Hours of illumination | Mgchl/gm fresh weight |      |       | $\mu$ g carotenoid/gm fresh weight |      |       |
|-----------------------|-----------------------|------|-------|------------------------------------|------|-------|
|                       | Control               | AT   | AT    | Control                            | AT   | AT    |
|                       |                       | 5 mM | 10 mM |                                    | 5 mM | 10 mM |
| 0                     | 0.10                  | 0.10 | 0.30  | 6                                  | 5    | 5     |
| 2                     | 0.27                  | 0.22 | ..    | 14                                 | 12   | 9     |
| 4                     | 0.57                  | 0.41 | 0.25  | 26                                 | 22   | 13    |
| 6                     | 0.84                  | 0.58 | 0.45  | 40                                 | 29   | 20    |
| 12                    | 1.71                  | 1.17 | 0.97  | 80                                 | 43   | 29    |
| 16                    | 1.90                  | 1.50 | 1.11  | 100                                | 48   | 33    |
| 20                    | 2.00                  | 1.53 | 1.18  | 120                                | 52   | 35    |
| 24                    | 2.03                  | 1.53 | 1.20  | 126                                | 60   | 35    |

TABLE II

Effect of amitrole on the chlorophyll synthesis in excised leaves

Two sets of excised leaves from 7-day old dark grown seedlings of *Phaseolus radiatus* were floated in distilled water and 5 mM amitrole separately and were illuminated with a bank of light. Leaf samples were removed at indicated time intervals and their chlorophyll content estimated.

| Hours of illumination | $\mu$ g chlorophyll/gram fresh weight |          |
|-----------------------|---------------------------------------|----------|
|                       | Control                               | Amitrole |
| 0                     | 50                                    | 50       |
| 2                     | 101                                   | 75       |
| 4                     | 158                                   | 108      |
| 6                     | 197                                   | 110      |
| 8                     | 242                                   | 106      |
| 10                    | 279                                   | 110      |
| 12                    | 330                                   | 109      |
| 14                    | 365                                   | 115      |

all our experiments, concentrations of less than 20 mM amitrole were employed.

The effect of amitrole on the chloroplast development in excised dark grown leaves exposed to light is shown in Table II. There was a rapid synthesis of chlorophyll in dark grown, excised leaves floated in distilled water during illumination. The chlorophyll content increased 7-fold within 14 hours of illumination. There was only a 2-fold increase during the same period when the leaves were

floated in 5 mM aminotriazole. This amounted to nearly 70% of inhibition as compared to control leaves. The effectiveness of amitrole in causing maximal inhibition of chloroplast development when applied directly to the leaves and not through the roots is obviously due to the limited absorption and translocation in the latter case.

The carotenoid synthesis in the leaves of amitrole-treated seedlings after exposure to light is compared with the leaves of control seedlings as shown in Table I. The carotenoid content reached the maximal level of 125  $\mu$ g per gram fresh weight in control leaves within 24 hours. The levels of carotenoid in amitrole-treated seedlings were, however, reduced to 60 and 35  $\mu$ g per gram fresh weight, depending on the concentration of amitrole administered. The effect of amitrole on carotenoid biosynthesis was more pronounced than that of chlorophyll synthesis.

Diverse metabolites like aminoacids, bases and vitamins were shown to annul or nullify the toxic effect of amitrole when applied concurrently with or later of its application in organisms like unicellular algae, yeast and bacteria which has led to the speculation that amitrole might interfere with their biosynthesis. In order to ascertain whether similar reversal of the inhibitory effect of amitrole on chloroplast development could be achieved, a systematic study was made with several compounds, that were shown to annul its effect by others, on their ability to reverse the bleaching effect of amitrole in the leaves of *Phaseolus* seedlings. The effect of equimolar concentrations of various metabolites in reversing the amitrole-induced inhibition of chlorophyll synthesis is summarised in Table III. Among the aminoacids tried, L-histidine, glycine and sodium succinate mixture (as substrates for  $\delta$ -aminolevulinate synthesis), L-leucine and L-cysteine were found to reverse completely the inhibitory action of amitrole on chloroplast development; while the aminoacids like DL-glutamic acid, DL-serine and glycine were comparatively less effective in retarding the inhibition caused by amitrole. DL-phenylalanine, lysine and L-cystine, on the other hand, were found to have practically no effect. Riboflavin was the other substance tried and found to be effective in annulling the inhibitory action of amitrole.

Similarly, the effect of certain metal ions and the chelating agent, ethylene diamine tetra-acetic acid (EDTA) on reversing the effect of amitrole on chloroplast development as measured by chlorophyll content is shown in Table IV. Among the metal ions tried  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$  were very effective in nullifying the action of amitrole while  $\text{Ca}^{2+}$  and  $\text{Na}^+$  had no effect at all. EDTA when applied along with amitrole annulled the inhibitory action of the compound completely.

TABLE III

Effect of various aminoacids on the inhibitory action of amitrole on the chlorophyll content of *Phaseolus* seedlings

Dark grown 6-day old seedlings were treated either with 5 mM amitrole separately or along with an equimolar concentration of aminoacids. The treated seedlings were kept in the dark for 24 hours. The pretreated seedlings were then exposed to light for a period of 24 hours and total chlorophyll and carotenoid levels estimated at the end of the period. The figures given are the average of three different experiments.

| Treatment          | Total chlorophyll<br>$\mu\text{g/gm}$ fresh weight | % Control | Total carotenoid<br>$\mu\text{g/gm}$ fresh wt. | % Control |
|--------------------|--|-----------|--|-----------|
| None               | 1250   | 100.00    | 198  | 100.00    |
| Amitrole           | 550  | 44.00     | 80   | 40.40     |
| +Riboflavin        | 1263   | 102.54    | 200  | 101.00    |
| +Glycine           | 1200   | 96.00     | 197  | 99.49     |
| +Sodium succinate  |  |           |  |           |
| +L-Histidine       | 1275   | 102.00    | 196  | 98.98     |
| +L-Leucine         | 1120   | 89.60     | 189  | 95.45     |
| +L-Cysteine        | 1100   | 88.00     | 179  | 90.40     |
| +DL-Serine         | 890  | 71.20     | 160  | 80.80     |
| +DL-Glutamic acid  | 908  | 72.60     | 158  | 79.79     |
| +Glycine           | 785  | 62.60     | 141  | 71.21     |
| +DL-Alanine        | 540  | 42.80     | 86   | 43.33     |
| +DL-Phenyl-alanine | 533  | 43.20     | 83   | 41.91     |
| +Lysine            | 510  | 40.80     | 80   | 40.40     |
| +L-Cystine         | 500  | 40.00     | 76   | 38.38     |

#### Discussion

Among the various herbicides which are known to inhibit the photosynthetic function of the leaves, amitrole was found to affect the chloroplast development specifically and completely without any significant effect on the rest of the cellular metabolism<sup>7</sup>. From the data presented here, it can be seen that when amitrole-treated dark grown leaves were exposed to light both chlorophyll and carotenoid developments are inhibited. Since the two pigments are not biosynthetically interrelated, the mechanism for the concurrent inhibition of both chlorophyll and carotenoids cannot possibly be due to the direct action of amitrole on their biosynthesis. This is substantiated by the fact that the enzymes involved in the biosynthesis of prophyrin or heme were not inhibited by amitrole under *in vitro* con-

TABLE IV

Effect of various metal ions on the inhibitory action of amitrole on the chlorophyll content of *Phaseolus* seedlings

The experimental details are as in Table III.

| Treatment                 | Total chlorophyll<br>$\mu\text{g/g}$ fresh weight | % Control | Total carotenoid<br>$\mu\text{g/gram}$ fresh wt. | % Control |
|---------------------------|---|-----------|--|-----------|
| None                      | 1250  | 100.00    | 198  | 100.00    |
| Amitrole                  | 550   | 44.00     | 80   | 40.40     |
| +Ferric chloride (1 mM)   | 1272  | 102.54    | 195  | 98.98     |
| +Magnesium chloride (1mM) | 1189  | 95.12     | 187  | 95.45     |
| +Calcium chloride         | 545   | 43.60     | 83   | 41.91     |
| +Sodium phosphate         | 505   | 50.50     | 81   | 40.90     |
| +EDTA (0.5 mM)            | 1243  | 102.54    | 200  | 100.00    |

ditions<sup>8,9</sup>. Neither were there evidences for the accumulation of specific intermediates of chlorophyll in amitrole-treated cells. There was also no generalized reduction in the levels of all porphyrin containing compounds in amitrole-treated cells<sup>9</sup>.

Though accumulation of phytoene—a carotenoid precursor—in amitrole-treated cells is reported, it is not known whether it is the breakdown product of carotenoids<sup>10</sup>, or due to an inhibition of further conversion of this intermediate<sup>11</sup>. Phytoene accumulation itself has not been confirmed by others. Among the various biochemical compositions compared between normal and amitrole-bleached cells, total lipid was found to be drastically reduced besides the pigments in amitrole-bleached cells<sup>12</sup>. Considering all these facts, it is reasonable to conclude that the observed decrease in the levels of the two pigments may be due to interference in the development of the chloroplast structure preventing the accumulation of these pigments rather than interfering with their syntheses *per se*.

The reversal of amitrole inhibition of chloroplast development by a set of highly unrelated compounds such as a few aminoacids, riboflavin, purines and certain metal ions<sup>13</sup> at equimolar concentration indicate that this herbicide is not affecting the chloroplast development either by interfering with the syntheses of these pigments or with the metabolism of these compounds directly. It is likely that these

compounds would complex with aminotriazole to a varying level and thereby either prevent its absorption into the cell or detoxify it or its derivatives inside the cell. In fact, it has been shown that amitrole forms complexes with proteins or amino-acids<sup>14-16</sup>.

Similarly, the fact that certain metal ions like  $Fe^{3+}$  and  $Mg^{2+}$  at equimolar concentrations could also reverse its adverse effects (Table IV), indicates that amitrole possibly acts as a chelator to interfere with the normal utilization of  $Fe^{3+}$  or  $Mg^{2+}$  in the synthesis of proteins like cytochromes or pigments which in turn would effect the formation of lamellar membrane. The evidence in favour of this comes from the fact that these ions were found to reverse the observed immediate inhibition of the photosynthetic and respiratory oxygen exchange reactions by amitrole<sup>12</sup>. Apparently amitrole seems to be very specific in complexing with certain metal ions, since the calcium and sodium ions have no such reversal effect on the herbicidal action of amitrole (Table IV).

In conclusion it can be seen from the data presented here that the observed low levels of carotenoid and chlorophyll pigments are only an effect on the development of chloroplast structure rather than its direct interference with their biosynthesis. Similarly the reversal of amitrole inhibition by several organic compounds may be due to their ability to complex with amitrole thereby either preventing its entry into cells or detoxify it inside the cells. The fact that certain metal ions could also reverse the amitrole effect substantiates this possibility.

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#### ALL-INDIA CONGRESS OF ZOOLOGY

The Third All-India Congress of Zoology sponsored by the Zoological Society of India will be held under the auspices of the Andhra University, Waltair, from December 29, 1975 to January 2, 1976. Besides meetings in eleven sections, symposia will be held on the following : Zoology and Society,

Wild Life Conservation in India, Zoology in Industry, and Present Concepts of Modern Zoology. For details write to : Dr. B. K. Behura, General Secretary, Department of Zoology, Utkal University, Bhubaneswar 751 004.



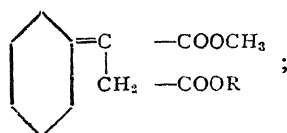
## LETTERS TO THE EDITOR

SYNTHESIS OF 1, 1-CYCLOHEXYLIDENE-  
1, 2-DICARBOXY-3-ARYL PROPADIENES

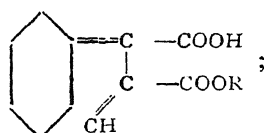
In course of studies in the nonaromatic  $C_6-C_3-C_6$  systems, 1, 1-cyclohexylidene-3-aryl-1, 2-dicarboxy-propadienes were prepared.

Johnson *et al.*<sup>1</sup> had observed the formation of a mixture of cyclohexenyl and cyclohexylidene succinic acids in Stobbe reaction of cyclohexanone and dimethyl succinate. Subsequently<sup>2</sup>, it was shown that the primary product of the reaction was the cyclohexylidene system when the milder condition of short period at room temperature was used. Methyl  $\alpha$ ,  $\alpha$ -cyclohexylidene succinate acid ester (1 *a*) (m.p. 96-97° C) was prepared by this procedure<sup>2</sup> and the diester (1 *b*) obtained by esterification with diazomethane

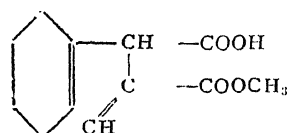
Condensation of the diester (1 *b*) with anisaldehyde in the presence of potassium tert butoxide at room temperature gave 1, 1-cyclohexylidene-1-carboxy-2-carbomethoxy-3-*p*-methoxyphenyl propadiene (2 *a*) (m.p. 118-119°); U.V.  $\lambda_{\max}^{EtOH}$  308 n.m., (log  $\epsilon$  4.07);



(1 *a*): R=H  
(1 *b*): R=CH<sub>3</sub>



(2 *a*): X=OMe, R=Me  
(2 *b*): X=OMe, R=H  
(3 *a*): X=H, R=Me  
(3 *b*): X=H, R=H



(4)

NMR spectrum for ester-acid (2 *a*) showed  $A_2X_2$  type, characteristic of many para-disubstituted benzenes; (CDCl<sub>3</sub>,  $\delta$ ): 1.52 (6H, *s*, cyclohexane ring), 2.04 (2H, *s*, cyclohexane ring), 2.86 (2H, *s*, cyclohexane ring), 3.78 (6H, 2x OCH<sub>3</sub>), 6.77-6.92 (2H, *d*, J = 9 Hz ArH), 7.27-7.44 (2H, *d*, J = 9 Hz, ArH). For ester-acid (3 *a*); (CDCl<sub>3</sub>,  $\delta$ ): 1.47 (6H, *s*, cyclohexane ring), 2.00 (2H, *s*, cyclohexane ring), 2.84 (2H, *s*, cyclohexane ring), 3.80 (3H, *s*, OCH<sub>3</sub>), 7.27 (5H, *s*, C<sub>6</sub>H<sub>5</sub>). The absence of an olefinic proton (blank 4-7  $\delta$ ) supports the structure (3 *a*) instead of (4). The separation of the two methylenes at 2.00 and 2.84  $\delta$  were attributed to the shift of one of the pair at position  $\beta$  of cyclohexane ring due to the shielding of electronegative carboxyl group at C<sub>1</sub>. This was further supported by the NMR spectrum of acid-ester (1 *a*); (CDCl<sub>3</sub>,  $\delta$ ): 1.62 (6H, *s*, cyclohexane ring), 2.25 (2H, *s*, cyclohexane ring), 2.65 (2H, *s*, cyclohexane ring), 3.40 (2H, *s*, CH<sub>2</sub>-aliphatic), 3.70 (3H, *s*, OCH<sub>3</sub>).

Saponification of the acid-esters (2 *a*) and (3 *a*) gave the respective diacids (2 *b*) and (3 *b*).

I.R. 2950 (C-H stretch of aromatic ring), 1716 conjugated ester), 1693 (Ph conjugated  $\alpha$ ,  $\beta$ -unsaturated acid), 1609 (Ph-C=C), 1263 (C-O stretch), 1177 (-O-ether stretch), 830  $cm^{-1}$  (1:4 disubstituted benzene ring). Condensation with benzaldehyde gave the corresponding phenyl compound (3 *a*) (m.p. 110-11°); U.V.  $\lambda_{\max}^{EtOH}$  282 n.m. (log  $\epsilon$  4.14); I.R. 2948, 1715, 1687 1606, 1255  $cm^{-1}$  and the distinct hypsochromic shift of 26 n.m. on lack of the *p*-OMe group (a shift of 25 n.m. is noted for a *p*-methoxy substituent in ArCOR systems<sup>3</sup>) suggested that the double bond was in conjugation with the arylidene unit thus indicating the structure (3 *a*) and not the cyclohexenyl system (4).

## Experimental

1,1-Cyclohexylidene-1-carboxy-2-carbomethoxy-3-phenyl propadiene (3 *a*).—A mixture of 1, 1-cyclohexylidene dimethyl succinate (1 *b*) (2.0 gm.) and benzaldehyde (0.94 gm.) was added to a well stirred solution of tert. butanolic potassium tert. butoxide prepared by dissolving potassium (0.36 gm) in dry tert. butanol (11 ml). After stirring for one hour at room temperature and in dry inert conditions, the reaction mixture was cooled in ice and acidified with 3N-hydrochloric acid. The alcohol was distilled off under reduced pressure, the reaction products extracted with ether in cold and the acidic substance was separated using

ice cold 10% sodium carbonate solution, which on acidification gave 1, 1-cyclohexylidene-1-carboxy-2-carbomethoxy-3-phenyl propadiene (3 a) (2.39 gm); crystallised from benzene-petroleum ether, m.p. 110-111°; found: eq. wt. 297.6; C, 72.20; H, 6.72. Required for  $C_{18}H_{20}O_4$ : eq. wt. 300.3; C, 72.01; H, 6.71%. U.V.  $\lambda_{\max}^{EtOH}$  282 n.m. (log  $\epsilon$  4.14); I.R. 2948, 1715, 1687, 1606, 1255  $cm^{-1}$ .

1, 1-Cyclohexylidene-1-carboxy-2-carbomethoxy-3-p-methoxyphenyl propadiene (2 a).—Similarly 1, 1-cyclohexylidene dimethyl succinate (1 b) (2.6 gm) with anisaldehyde (1.2 gm) in potassium tert. butoxide solution (11 ml) gave 1, 1-cyclohexylidene-1-carboxy-2-carbomethoxy-3-p-methoxyphenyl propadiene (2 a) (2.66 gm); crystallised from benzene-petroleum ether, m.p. 118-119°; found: eq. wt. 343.4; C, 69.36; H, 6.64. Required for  $C_{19}H_{22}O_5$ : eq. wt. 330.4; C, 69.07; H, 6.71%. U.V.  $\lambda_{\max}^{EtOH}$  308 n.m. (log  $\epsilon$  4.07); I.R. 2950, 1716, 1693, 1609, 1263, 1177, 830  $cm^{-1}$ .

1, 1-Cyclohexylidene-1, 2-dicarboxy-3-phenyl propadiene (3 b).—1, 1-Cyclohexylidene-1-carboxy-2-carbomethoxy-3-phenyl propadiene (3 a) on saponification with 8% alc. KOH solution when refluxed for eight hours gave 1, 1-cyclohexylidene-1, 2-dicarboxy-3-phenyl propadiene. Crystallised from ethanol-water; m.p. 213-214°; found: eq. wt. 145.5; C, 71.50, H, 6.38. Required for  $C_{17}H_{18}O_4$ : eq. wt. 143.1; C, 71.31; H, 6.33%. U.V.  $\lambda_{\max}^{EtOH}$  258 n.m. (log  $\epsilon$  4.10); I.R. (nujol) 1695 ( $\alpha$ ,  $\beta$ -unsaturated acid), 1675  $cm^{-1}$  (cinnamic acid unit).

1, 1-Cyclohexylidene-1, 2-dicarboxy-3-p-methoxyphenyl propadiene (2 b).—1, 1-cyclohexylidene-1-carboxy-2-carbomethoxy-3-p-methoxyphenyl propadiene (2 a) on saponification with 8% alc. KOH gave 1, 1-cyclohexylidene-1, 2-dicarboxy-3-p-methoxyphenyl propadiene (2 b). Crystallised from ethanol-water; m.p. 240-243°; found: eq. wt. 168.6; C, 68.21; H, 6.18. Required for  $C_{18}H_{20}O_5$ : eq. wt. 158.2; C, 68.36; H, 6.37%. U.V.  $\lambda_{\max}^{EtOH}$  297 n.m. (log  $\epsilon$  3.94).

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## MICROBIOCIDAL ACTIVITIES OF SOME AMIDE COMPLEXES OF DIPHENYL TIN DICHLORIDE

IN contrast to tin and its inorganic compounds, organotin compounds are reported to possess noteworthy antimicrobial and other biologically important properties. Several reports have revealed that many organometallic compounds of tin<sup>1-5</sup> are effective biocidal agents. In order to assess the effect of covalency and size of the organometallic molecule, molecular addition compounds of diphenyltin (IV) dichloride, with certain amides, were evaluated *in vitro* for their microbiocidal propensities against some fungi and bacteria.

### Experimental

$(C_6H_5)_2SnCl_2$  (diphenyltin dichloride) and complexes of this Lewis acid with dimethylformamide, dimethylacetamide and diphenylacetamide were synthesised by methods reported earlier<sup>6-9</sup>. The amides of Analar grade (BDH) were used without further purification. All the test chemicals being soluble in acetone, acetonic solutions were used for making suitable concentrations.

Five species of fungi, *Aspergillus niger* Van Tieghem, *Candida albicans* (Robin) Berkhout, *Cryptococcus neoformans* (Sanfelice) Vuillemin, *Microsporum canis* Bodin and *Trichophyton mentagrophytes* (Robin) Blanchard and four bacterial species, viz., *Bacillus subtilis* Cohn emend. Prazmowski, *Escherichia coli* (Migula) Castellani and Chalmers, *Salmonella typhi* Warren and Scott and *Staphylococcus aureus* Rosenback were used in the present investigation.

The activity of the chemicals was evaluated against the test-organisms by determining the lowest concentration required for the growth inhibition (of the micro-organisms) upto a maximum concentration of 100  $\mu g/ml$ . The methods employed by Srivastava *et al.*<sup>3-5</sup> were used for this study and the results are given in Table I.

Diphenyltin dichloride completely arrested the growth of *A. niger*, *T. mentagrophytes*, *B. subtilis* and *S. aureus* at 25, 25, 25 and 6.25  $\mu g/ml$ , respectively, while the compound was ineffective against the rest. Dimethylacetamide showed a fungicidal potential controlling the growth of *A. niger*, *C. neoformans*, and *T. mentagrophytes* at the concentration of 25  $\mu g/ml$  while the activity of other Lewis bases, dimethylformamide and diphenylacetamide, was practically non-existent against the test-organisms. The anti-microbial behaviour of  $(C_6H_5)_2SnCl_2$ , on complexation with the test Lewis bases (i.e., the expansion of covalency of the tin atom and the molecule size) has increased because, the complexes generally arrested the growth

TABLE I

Minimal inhibitory concentration ( $\mu\text{g/ml}$ ) of the test compounds against some fungi and bacteria

| Compound                                     | Fungi           |                    |                      |                          |                 | Bacteria           |                  |                 |                |
|--|-----------------|--------------------|----------------------|--------------------------|-----------------|--------------------|------------------|-----------------|----------------|
|  | <i>A. niger</i> | <i>C. albicans</i> | <i>C. neoformans</i> | <i>T. mentagrophytes</i> | <i>M. canis</i> | <i>B. Subtilis</i> | <i>S. aureus</i> | <i>S. typhi</i> | <i>E. coli</i> |
| $(\text{C}_6\text{H}_5)_2\text{SnCl}_2$      | 25              | >100               | >100                 | 25                       | >100            | 25                 | 6.25             | > 00            | >100           |
| DMF  | >100            | >100               | >100                 | >100                     | >100            | >100               | >100             | >100            | >100           |
| DMA  | 25              | >100               | 25                   | 25                       | ..              | >100               | >100             | >100            | >100           |
| DPA  | >100            | >100               | >100                 | >100                     | ..              | >100               | >100             | >100            | >100           |
| $(\text{C}_6\text{H}_5)_2\text{SnCl}_2$ 2DMF | ..              | >100               | >100                 | 6.25                     | 3.12            | 6.25               | 6.25             | >100            | >100           |
| $(\text{C}_6\text{H}_5)_2\text{SnCl}_2$ 2DMA | 1.25            | >100               | 25                   | 12.5                     | ..              | 12.5               | 6.25             | >100            | >100           |
| $(\text{C}_6\text{H}_5)_2\text{SnCl}_2$ DPA  | 25              | >100               | 25                   | >100                     | ..              | 12.5               | 6.25             | >100            | >100           |

$(\text{C}_6\text{H}_5)_2\text{SnCl}_2$  = diphenyltin (IV) dichloride  
 DMF = dimethylformamide  
 DMA = dimethylacetamide and  
 DPA = diphenylacetamide.

8. Bajpai, Beena, *Ph.D. Thesis*, Lucknow University, 1973.

9. Srivastava, T. N., Tandon, S. K. and Bajpai, B., *Inorganica Chimica Acta* (In press).

of all the fungi, at considerably lower levels (except *C. albicans*) than the uncomplexed  $(\text{C}_6\text{H}_5)_2\text{SnCl}_2$ . This enhancement in the microbiocidal property was also evident against *B. subtilis*, but other bacteria remained unaffected by the complexes.

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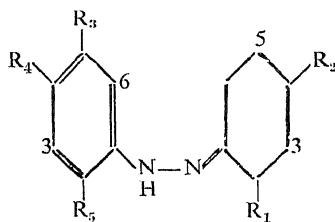
### PMR STUDIES ON SUBSTITUTED TETRAHYDROCARBAZOLES

NECESSITY of an easy approach for the structural assignment of cyclised hydrazones was felt during the course of our studies on the synthesis of biodynamic hydrazones<sup>1</sup> and tetrahydrocarbazoles<sup>2</sup>. PMR studies of these compounds indicated it to be a useful tool for the structural assignment of these analogs and homologs. The results of these studies along with their IR spectra are reported in this communication.

4-Bromo-2-nitro, 5-chloro-2-nitro, 4-nitro and 4, 5-dichloro-2-nitrophenylhydrazines on condensation with cyclohexanone, 2-methyl and 4-methyl-cyclohexanones in alcohol and glacial acid gave the corresponding hydrazones (I-XII, Table I) in good yields. Similar condensation of 2, 3-dichlorophenylhydrazine hydrochloride and cyclohexanone in alcohol with sodium acetate as the condensing agent afforded cyclohexanone-(2, 3-dichlorophenyl) hydrazine (XIII) in 70% yield, m.p. 152° (Found: C 55.85, H 5.23 and N 10.62;  $\text{C}_{12}\text{H}_{14}\text{Cl}_2\text{N}_2$  requires C 56.03, H 5.45 and N 10.89%); IR (KBr) 3300, 2870, 2800, 1615, 1575, 1470, 890, 860 and 760  $\text{cm}^{-1}$  taken on Perkin-Elmer 337 grating instrument.

A mixture of XIII and concentrated hydrochloric acid in the ratio 1:10 when heated on a water bath for an hour afforded 7, 8-dichloro-1, 2, 3, 4-tetrahydrocarbazole (XXI), m.p. 205° (Found: C 59.89, H 4.32 and N 5.56;  $\text{C}_{12}\text{H}_{11}\text{Cl}_2\text{N}$  requires C 60.0, H 4.59

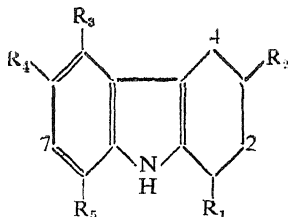
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TABLE I  
Substituted hydrazones

| Compd. No. | R <sub>1</sub>  | R <sub>2</sub>  | R <sub>3</sub> | R <sub>4</sub>  | R <sub>5</sub>  | C <sub>2</sub> -CH <sub>3</sub> | C <sub>4</sub> -CH <sub>3</sub> | H-2                | H-3                | H-4                 | H-5                 | H-6                |
|------------|-----------------|-----------------|----------------|-----------------|-----------------|---------------------------------|---------------------------------|--------------------|--------------------|---------------------|---------------------|--------------------|
| I          | H               | H               | H              | Br              | NO <sub>2</sub> | ..                              | ..                              | ..                 | 8.05,d,<br>J = 2   | ..                  | 7.33,dd,<br>J = 2.9 | 7.77,d,<br>J = 9   |
| II         | CH <sub>3</sub> | H               | H              | Br              | NO <sub>2</sub> | 1.21,d,<br>J = 6                | ..                              | ..                 | 8.05,d,<br>J = 2   | ..                  | 7.38,dd,<br>J = 2.9 | 7.81,d,<br>J = 9   |
| III        | H               | CH <sub>3</sub> | H              | Br              | NO <sub>2</sub> | ..                              | 0.96,d,<br>J = 5.5              | ..                 | 8.08,d,<br>J = 2   | ..                  | 7.38,dd,<br>J = 2.9 | 7.81,d,<br>J = 9   |
| IV         | H               | H               | Cl             | H               | NO <sub>2</sub> | ..                              | ..                              | ..                 | 7.98,d,<br>J = 9   | 6.65,dd,<br>J = 2.9 | ..                  | 7.75,d,<br>J = 2   |
| V          | CH <sub>3</sub> | H               | Cl             | H               | NO <sub>2</sub> | 1.21,d,<br>J = 6                | ..                              | ..                 | 8.0,d,<br>J = 9    | 6.65,dd,<br>J = 2.9 | ..                  | 7.76,d,<br>J = 2   |
| VI         | H               | CH <sub>3</sub> | Cl             | H               | NO <sub>2</sub> | ..                              | 0.98,d,<br>J = 5.5              | ..                 | 8.02,d,<br>J = 9   | 6.66,dd,<br>J = 2.9 | ..                  | 7.81,d,<br>J = 2   |
| VII        | H               | H               | H              | NO <sub>2</sub> | H               | ..                              | ..                              | 7.00,d,<br>J = 9   | 8.08,d,<br>J = 9   | ..                  | 8.08,d,<br>J = 9    | 7.00,d,<br>J = 9   |
| VIII       | CH <sub>3</sub> | H               | H              | NO <sub>2</sub> | H               | 1.18,d,<br>J = 6                | ..                              | 6.96,d,<br>J = 9   | 8.04,d,<br>J = 9   | ..                  | 8.04,d,<br>J = 9    | 6.96,d,<br>J = 9   |
| IX         | H               | CH <sub>3</sub> | H              | NO <sub>2</sub> | H               | ..                              | 1.00,d,<br>J = 5.5              | 7.00,d,<br>J = 9.5 | 8.05,d,<br>J = 9.5 | ..                  | 8.05,d,<br>J = 9    | 7.00,d,<br>J = 9.5 |
| X          | H               | H               | Cl             | Cl              | NO <sub>2</sub> | 1.21,d,<br>J = 6                | ..                              | ..                 | 8.16,s             | ..                  | ..                  | 7.91,s             |
| XI         | CH <sub>3</sub> | H               | Cl             | Cl              | NO <sub>2</sub> | ..                              | 1.00,d,<br>J = 5.5              | ..                 | 8.15,s             | ..                  | ..                  | 7.90,s             |
| XII        | H               | CH <sub>3</sub> | Cl             | Cl              | NO <sub>2</sub> | ..                              | ..                              | ..                 | 8.20,s             | ..                  | ..                  | 7.95,s             |

and N 5.83%). Similar cyclization afforded tetrahydrocarbazoles<sup>3-4</sup> XIV-XX (Table II). The IR spectral data with characteristic bands around 3350 (N-H stretching vibrations), 2900, 2800 (C-H stretching), 1610 (Ar) 1565, 1330 (Ar-NO<sub>2</sub>), 830 (1, 2, 4-trisubstituted benzene)

680-740 [C-X (Halogen)], 1150-1350 twisting and wagging vibrations due to methylenes and 870 cm<sup>-1</sup> (C-N stretching vibrations for nitroaromatic compounds) frequencies are compatible with the assigned structures,

TABLE II  
Substituted tetrahydrocarbazoles\*

| Compd.<br>No. | R <sub>1</sub>  | R <sub>2</sub> | R <sub>3</sub> | R <sub>4</sub>  | R <sub>5</sub>  | C <sub>1</sub> -CH <sub>3</sub> | C <sub>3</sub> -CH <sub>3</sub> | H-5              | H-6                | H-7                 | H-8              |
|---------------|-----------------|----------------|----------------|-----------------|-----------------|---------------------------------|---------------------------------|------------------|--------------------|---------------------|------------------|
| XIV           | H               | H              | H              | Br              | NO <sub>2</sub> | ..                              | ..                              | 7.65,d,<br>J = 2 | ..                 | 7.93,d,<br>J = 2    | ..               |
| XV            | H               | H              | Cl             | H               | NO <sub>2</sub> | ..                              | ..                              | ..               | 7.01,d,<br>J = 8.5 | 7.90,d,<br>J = 8.5  | ..               |
| XVI           | CH <sub>3</sub> | H              | Cl             | H               | NO <sub>2</sub> | 1.18,d,<br>J = 6                | ..                              | ..               | 7.01,d,<br>J = 8.5 | 7.90,d,<br>J = 8.5  | ..               |
| XVII          | H               | CH             | Cl             | H               | NO <sub>2</sub> | ..                              | 1.00,d,<br>J = 5.5              | ..               | 7.01,d,<br>J = 9   | 8.05,d,<br>J = 9    | ..               |
| XVIII         | H               | H              | H              | NO <sub>2</sub> | H               | ..                              | ..                              | 8.28 d,<br>J = 2 | ..                 | 7.96,dd,<br>J = 2.9 | 7.23,d,<br>J = 9 |
| XIX           | CH <sub>3</sub> | H              | H              | NO <sub>2</sub> | H               | 1.16,d,<br>J = 6                | ..                              | 8.40,d,<br>J = 2 | ..                 | 8.06,dd,<br>J = 2.9 | 7.30,d,<br>J = 9 |
| XX            | H               | H              | Cl             | Cl              | NO <sub>2</sub> | ..                              | ..                              | ..               | ..                 | 8.00,s              | ..               |

\* NMR spectra recorded on Varian A-60 D instrument using TMS reference and CDCl<sub>3</sub> as solvent. The chemical shifts are expressed in  $\delta$  units with J values in Hz.

Thanks are due to Dr. Nitya Anand (Director), Dr. R. S. Kapil, Dr. P. C. Jain of C.D.R.I., Lucknow, for analytical data and also to Dr. S. P. Gupta, Head of the Department, for his keen interest and valuable suggestions during the course of this work.

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#### A NOTE ON TRANSAMINASES IN THE RIPENING OF BANANAS ON STORAGE

TRANSAMINASES catalyze the reactions involving the transfer of an amino group from an  $\alpha$ -amino acid to an  $\alpha$ -keto acid. Leorara and Eurreis<sup>1</sup> reported the presence of transaminase activity in various plants and plant tissues. Schales and Schales<sup>2</sup> gave indirect evidence for the presence of transaminase in 42 different plants and plant organs. The present study reports the variation of aspartate-alanine amino-transferase during the ripening of different varieties of banana, viz., Basrai, Harichal, Lakel (variety of *Musa Cavendishii*), Rajeli, Safed velchi (variety of *Musa paradisiaca*) at 13°C.

In order to get banana bunches of uniform maturity, nearly 100 banana plants were tagged at the time of inflorescence emergence, in a nearby banana plantation. From these lots, two bunches each of uniform development were harvested at 100 days after the inflorescence

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emergence. The upper hands of these bunches were stored in an incubator at 13° C. The weight ratio of pulp to skin was determined to assess the maturity of the fruits. Three fingers from each of the five banana hands were removed at a time and the average values are reported.

With a view to finding out the solubility of transaminases, distilled water, 3% sodium chloride and phosphate buffer (pH 7.0, 0.1 M) were employed and the enzyme activity was determined by the method of Reitman and Frankel<sup>3</sup>. The phosphate buffer was the best medium for transaminases of the banana fruit pulp.

TABLE I

*Aspartate aminotransferase and alanine aminotransferase activities during the ripening of bananas*  
(Values expressed in sp. activity per 100 g fresh tissue)

| Variety      | Fractions | Storage period (days after harvesting) |      |      |      |           |
|--------------|-----------|--|------|------|------|-----------|
|              |           | 0                                      | 8    | 16   | 24   | 32        |
| Basrai       | I         | 1.35                                   | 1.61 | 1.65 | 1.76 | 3.00      |
|              | II        | 0.60                                   | 1.61 | 1.77 | 2.12 | 4.05      |
| Harichal     | I         | 0.30                                   | 1.34 | 1.33 | 2.63 | 2.95      |
|              | II        | 0.50                                   | 0.79 | 1.28 | 2.53 | 5.04      |
| Lalkel       | I         | 0.52                                   | 0.76 | 2.42 | 2.68 | 3.18      |
|              | II        | 0.51                                   | 1.05 | 2.12 | 3.00 | 3.48      |
| Rajeli       | I         | 0.50                                   | 1.04 | 2.44 | 4.39 | 4.88      |
|              | II        | 0.59                                   | 0.69 | 2.70 | 5.95 | 6.64      |
| Safed velchi | I         | 0.36                                   | 2.47 | 3.00 | 6.07 | Over ripe |
|              | II        | 0.47                                   | 0.91 | 3.04 | 4.30 |           |

Fraction I : Aspartate aminotransferase.

Fraction II : Alanine aminotransferase.

The banana pulp (15 g) was homogenised in a Waring blender with 50 ml of phosphate buffer for one hour at 5° C. The mass was squeezed through a muslin cloth and the filtrate centrifuged at 3000 r.p.m. for 20 minutes. The residue was re-extracted with 40 ml of the buffer and the combined extracts were again centrifuged after one hour. The supernatant layer was used as an enzyme source for the determination of aspartate-alanine transaminase. The assay procedure is based upon the fact that aspartate and alanine amino transferase enzyme, transfer amino group from amino acids, aspartic acid and alanine to keto acid, alpha-ketoglutaric acid forming respectively oxaloacetic acid and pyruvic acid which were colorimetrically estimated. Enzyme activity was expressed in terms of unit activity and defined as 1 µg of pyruvic acid formed in one hour and the specific activity was expressed as µg of pyruvic

acid formed per mg protein per hour. Protein was determined according to the method of Lowry *et al.*<sup>4</sup>.

It can be seen from Table I that aspartate amino transferase and alanine aminotransferase activities increased slowly in the early stages of ripening and then rapidly in the advanced progress of ripening. Giri *et al.*<sup>5</sup> reported that the transaminase activity increased on germination. Romani<sup>6</sup> also reported the presence of aspartate-alanine transaminase in the cortical tissue. In the present investigation, aspartate and alanine aminotransferase activities were found to be 0.26-6.07 and 0.47-6.64 unit activity respectively. At full ripe stage, the highest activity of aspartate aminotransferase was found in Safed velchi banana while alanine aminotransferase was highest in Rajeli banana. The increased activity of aspartate and alanine aminotransferase during storage and ripening of bananas demonstrate their important role of linking protein and organic acid metabolism.

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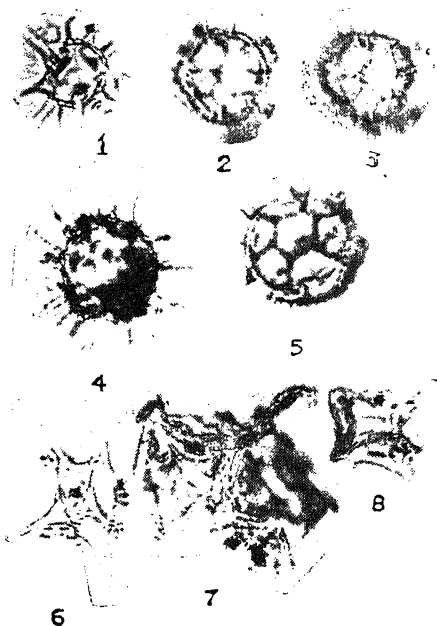
#### OCCURRENCE OF MICROPLANKTONS IN THE MIDDLE DEVONIAN ROCKS OF SASKATCHEWAN AND ALBERT, CANADA

In the course of microplankton studies of the Frasnian sequences of the interior plains of Canada, and to evaluate the genera and species of acritarchs and other microplanktons crossing the Middle-Upper Devonian boundary in the Saskatchewan and Alberta areas the author (Nautiyal, 1972<sup>1</sup>) analysed some samples from the Middle Devonian Elk Point Group of these regions for their microfaunal (microfloral) content. This report provides the first occurrence of acritarchs from the Middle Devonian sequences of Saskatchewan and Alberta (Figs. 1-8).

TABLE I

General stratigraphic succession of Middle Devonian units at Duval (6-18-36-6W3) well in Saskatoon, Saskatchewan, with organic-walled microplankton; well depth marks at formational boundaries, after Price and Ball<sup>3</sup>

| Frasnian sequences |                 |   | Well depth in feet |
|--------------------|-----------------|---|--------------------|
| GIVETIAN           | ELK POINT GROUP | DAWSON BAY FM.<br>Anhydrite, light olive gray, massive; Calcarenites, grey, fine to medium crystalline, argillaceous, occasionally pelletoidal, containing black, carbonaceous matter; <i>Cymatiosphaera perimembrana</i> , <i>Leiosphaeridia</i> sp. 1 types 1, 2, <i>L. microsaetosa</i> , <i>L. arbulata</i> , <i>L. papillata</i> and <i>Michrystidium</i> sp. 1. Shale, dark- or light-grey, invariably calcareous; <i>Leiosphaeridia</i> sp. 1, type 2. | 3,162              |
|                    |                 | "SECOND RED BEDS"<br>Mixture of greenish and reddish, calcareous or dolomitic shales containing black, carbonaceous matter; <i>Leiosphaeridia</i> sp. 1, type 2, <i>Michrystidium</i> sp.   | 3,241              |
|                    |                 | PRAIRIE EVAPORITE FM.<br>Halite, light olive grey and reddish orange, containing mixture of light olive grey to medium grey potash deposits and pale-red to green shale lenses; <i>Leiosphaeridia</i> sp. 1, <i>Michrystidium</i> sp. 2   | 3,274              |
|                    |                 |   | 3,545 (?)          |



FIGS. 1-8. Photomicrographs of acritarchs from Middle Devonian rocks at Bear Biltmore No. 1 (7-11-87-17W4) well of Alberta. Fig. 1. *Michrysti-*

The stratigraphic succession and the occurrence of microplanktons in the two wells examined in the present study are shown in Tables I and II.

The microplankton (acritarch) assemblages in the Middle Devonian sequences of Saskatchewan and Alberta are of very small in size (average about 20 microns), and characteristically simple, morphologically. In consequence, careful observation is needed in their study. They are important for the biostratigraphic correlation of the sequences. Their fair occurrence in the rocks needs further investigation. In addition, comparative studies of the microplankton assemblages in adjoining regions of the Canadian interior plains may be of value in the paleoenvironmental interpretation.

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dium sp. 1.; Figs. 2, 3. *Dictyotidium* sp. 1.; Fig. 4. *Baltisphaeridium* sp. 15; Fig. 5. *Cymatiosphaera perimembrana*; Fig. 6. *Veryhachium* sp. 2; Fig. 7. *Polyedryxium?* sp.; Fig. 8. *Polyedryxium* cf. *rabians*; Figs. 1-3, 5-7, from depth 2660'-2670'; Figs. 4, 8, from depth 1779'-1784'. All figs.,  $\times 3,000$  approx, but Fig. 7,  $\times 2,400$  approx.

TABLE II

General stratigraphic succession of Middle Devonian units at Bear Biltmore No. 1 (7-11-87-17W4) well in Alberta with organic-walled microplankton; formational nomenclature, after Norris<sup>2</sup>

| Frasnian sequences |   | Well depth<br>in feet |
|--------------------|---|-----------------------|
| GIVETIAN           | LIVOCK RIVER FM.  | 1,688                 |
|                    | "FIRST SALT FM."  |                       |
|                    | Dolomite and anhydrite, buff to light brown, with thin gypsum laminae; interbeds of buff and light brown calcarenite and grey siltstone; <i>Baltisphaeridium</i> sp. 15, <i>Cymatiosphaera</i> spp. 2, 3, <i>C. perimembrana</i> , <i>C. cf. tetraster</i> , <i>Leiosphaeridia</i> sp. 1 types 1, 2, <i>L. orbiculata</i> , <i>Micrhystridium</i> spp. 1, 2, <i>Multiplicisphaeridium rami-spinosum</i> , <i>Polyedryxium cf. rabians</i> , <i>Verhachium polyaster</i> types 1, 2, <i>V. cf. trispinosum</i> .   |                       |
|                    |   | 2,629 approx.         |
| ELK POINT GROUP    | METHY FM.   |                       |
|                    | Dolomite, grey to light brown, fine crystalline, with lenses of bituminous shales and carbonaceous matter; <i>Cymatiosphaera tetraster</i> , <i>Leiosphaeridia</i> sp. 1 type 2.  |                       |
|                    |   | 2,660 approx.         |
|                    | McLEAN RIVER FM.  |                       |
|                    | Dolomite, brown, fine crystalline, frequently porous; with interbeds of grey shales and brownish grey anhydrites; <i>Baltisphaeridium</i> spp. 15, 16, <i>Cymatiosphaera cf. cornifera</i> , <i>C. perimembrana</i> , <i>C. tetraster</i> , <i>Dictyotidium</i> sp. 1, <i>Leiofusa</i> sp. 1, <i>Leiosphaeridia</i> sp. 1 types 1, 2, <i>Micrhystridium</i> spp. 1, 2, 3, <i>M. angustum</i> , <i>M. bistchoensis</i> , <i>M. stellatum</i> , <i>Polyedryxium?</i> sp., <i>P. deflandrei?</i> , <i>Verhachium</i> sp. 14, <i>V. minor</i> , <i>V. polyaster</i> types 1, 2, <i>V. rhomboidium</i> , <i>V. cf. trispinosum</i> . |                       |
|                    |   | 2,863                 |

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# REPORT OF MICROPLANKTONS IN THE LOWER TRIASSIC ROCKS OF PAHLGAM, KASHMIR, INDIA

THERE are no records of microplanktons from the Triassic sequences of Kashmir, India. The present paper deals with the discovery of microplanktons (acritarchs and tasmaitifias) from the Lower Triassic rocks of Pahlgam (Kashmir). Their discovery in these rocks of the area is the first find of these groups of microfossils known to-date.

Up the western bank of Lidar valley in the Pahlgam (Kashmir) area, a stratigraphic section of micaceous shales with some limestone and siltstone layers is well exposed. About 60 metres thick part of this section,

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consisting mainly of shales (light to dark gray and black) with discontinuous limestone layers and siltstone bands, was studied and sampled within the area confined between the Coordinates  $34^{\circ} 1' 18''$  N to  $34^{\circ} 1' 30''$  N and Coordinates  $75^{\circ} 18' 36''$  E to  $75^{\circ} 18' 42''$  E. The rock samples were collected at about 6 metres interval throughout the stratigraphic section, constituting ten horizons P1 to P10.

The argillaceous rocks dominate the sequence. The black shales often break along the bedding plane and weather into light pale to cream coloured rock. Generally, the studied section reveals almost the same lithology and character of rocks that also appear in the Lower Triassic sequences exposed in other parts of the Pahlgam area (Middlemiss, 1909; Nakazawa *et al.*, 1970).

In the course of microfaunal (microfloral) investigation of these samples, the author came across with the fair occurrence of microplanktons (acritarchs and tasmanitids) associated with rich microflora of disaccate pollen grains. Their presence in the rocks is suggestive of further detailed work to be carried out on the sequences of similar age exposed in and around the area for biostratigraphic zonation and paleoecological study.

'INCERTAE SEDIS': Group—Acritarcha Evitt, 1963, Sub-group—Sphaeromorphitae Downie, Evitt and Sarjeant, 1963, Genus—*Leiosphaeridia* (Eis.), Downie and Sarjeant, 1963.

*Leiosphaeridia minuta* (Staplin) Downie and Sarjeant, 1953.—Test circular to subcircular; test wall laevigate, frequently folded; thin-walled; test diameter range 17 to 20  $\mu$ .

*Leiosphaeridia* cf. *L. wenlockia* Downie, 1959.—Test circular to subcircular; test wall laevigate and provided with a few folds; wall thickness about 1  $\mu$ ; test diameter range 22 to 45  $\mu$ .

*Leiosphaeridia* sp. 1.—Test sub-spherical; test wall smooth, often provided with a cryptosuture, wall thickness about 2.5  $\mu$ ; test diameter range 45 to 50  $\mu$ .

Sub-group—Tasmanititae (Sommer) Staplin *et al.*, 1955, Genus—*Tasmanites* (Newt.) Eisenack, 1958.

*Tasmanites* sp. 1.—Test spherical to sub-spherical; test wall provided with 2 to 3 folds, wall with indistinct micropores (about 0.5  $\mu$  in diameter), wall thickness about 3  $\mu$ ; test diameter range 87 to 100  $\mu$ .

*Tasmanites* sp. 2.—Test spherical; test wall without folds and provided with micropores (about 0.5  $\mu$  in diameter), wall thickness about 5  $\mu$ ; test diameter about 96  $\mu$ .

#### Distribution

The Pahlgam collection of the Lower Triassic sequence belongs to ten different horizons P1 to P10, in ascending order. All these horizons but P2 yielded microplanktons in varying quantitative composition (Fig. 1). These microfossils belong to two groups: acritarchs

and tasmanitids. Some horizons reveal their fair occurrence whereas the others have sporadic distribution.

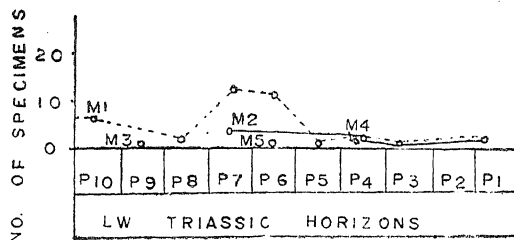


FIG. 1. Quantitative composition of organic-walled microplanktons (acritarch and tasmanitid) reported in the rocks representing each Lower Triassic horizons (P1 to P10) of Pahlgam. M1, *Leiosphaeridia minuta*; M2, *L. cf. wenlockia*; M3, *Leiosphaeridia* sp. 1; M4, *Tasmanites?* sp. 1; M5, *Tasmanites* sp. 2.

The medium gray shales with siltstone bands and lenticles (horizon P1) contain *Leiosphaeridia minuta*, and *L. cf. wenlockia* (Fig. 1). The light gray shales (horizon P3) also contain *Leiosphaeridia minuta*, and *L. cf. wenlockia*. In contrast, the medium gray shales with carbonaceous matter (horizon P4) reveal *Leiosphaeridia minuta*, *L. cf. wenlockia* and *Tasmanites* sp. 1. The medium gray limestones (horizon P5) show poor concentration of *Leiosphaeridia minuta*. The dark gray shales with rich carbonaceous matter (horizon P6) yielded a fair occurrence of *Leiosphaeridia minuta* and *Tasmanites* sp. 2. The light olive gray shale (horizon P7) contains fair concentration of *Leiosphaeridia minuta*, and *L. cf. wenlockia*. Black shales rich in pyrite grains (horizon P8) yielded *Leiosphaeridia minuta*. The light gray shales (horizon P9) shows *Leiosphaeridia* sp. 1. The light gray siltstones with gray shale bands (horizon P10) disclosed a fair occurrence of *Leiosphaeridia minuta*.

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LETHAL AND MUTAGENIC EFFECTS OF  
GAMMA RAYS ON *CHAETOMIUM AUREUM*  
CHIVERS.

GENUS *Chaetomium* has been little studied from the genetic point of view, excepting that some data on saltation and mutation<sup>1-3</sup> were reported earlier without, however, attaching particular importance to the cellulose

mutants possibly indicate the occurrence of mutation in various gene loci leading to a block in the synthesis of essential metabolites which might be required for the biosynthetic activities of alternate pathways. Morphological mutants of colonial, pigmented and apigmented mycelial types were induced at a rather low level of 0.92; with a dose of 40 Kr (Table I).

TABLE I  
*Lethal and mutagenic effects of gamma rays of Chaetomium aureum*

| Treatment<br>(Kr) | Average<br>surviving<br>ascospores/ml<br>$\times 10^6$ | Survival<br>%, | Colonies<br>tested | % Mutation    |             | Total |
|-------------------|--|----------------|--------------------|---------------|-------------|-------|
|                   |  |                |                    | Morphological | Biochemical |       |
| 0 (Control)       | 105.80   | 100.00         | 1080               | nil           | nil         | nil   |
| 20                | 44.59  | 42.14          | 1080               | 0.46          | nil         | 0.46  |
| 30                | 15.02  | 14.19          | 1080               | 0.46          | 1.38        | 1.84  |
| 40                | 6.96   | 6.57           | 1080               | 0.92          | 0.92        | 1.84  |
| 50                | 2.03   | 1.91           | 1080               | 0.46          | 2.31        | 2.77  |
| 60                | 1.65   | 1.55           | 1080               | 0.46          | 6.94        | 7.40  |
| 70                | 0.50   | 0.47           | 1080               | ..            | 1.85        | 1.85  |

Temperature of irradiation — 25° C.

decomposing character. Considering the importance of the enzyme cellulase in medicine, industry and agriculture an attempt was made to study the effect of gamma rays on *Chaetomium aureum* with reference to its physiological and biochemical behaviour. 15 ml of ascospore suspensions in aluminium capsules were subjected to gamma irradiation from 60 Co, the dosages being 20, 30, 40, 50, 60 and 70 Kr. Irradiated spores were plated on P-complete medium having 0.7% sorbose<sup>4</sup>. Survival per cent was determined after incubation at 30° C for five days. The average of 10 plates per treatment including the control was taken into consideration and characterisation of morphological and nutritional mutants were done on minimal medium<sup>5,6</sup> following the usual technique as adopted by Mitra and Chaudhuri<sup>6,7</sup>. Cellulose activity of different mutants and the wild strain was determined according to the standard techniques<sup>8-11</sup>.

Figure 1 shows that treatment with gamma rays gave a sharp decline in the survival per cent. The highest percentage of 6.94 biochemical mutants was observed with 60 Kr treatment, the different types obtained being vitamin, amino acid and nucleic acid requiring species (Table II). Most of the vitamin mutants were Paba requiring ones and others were deficient in inositol, nicotinic acid and Ca-pantothenate. Lysine, leucine and tryptophan requiring amino acid mutants were also observed in the irradiated population along with the nucleic acid mutants deficient in guanine, xanthine, adenine and hypoxanthine. Multiple growth factor

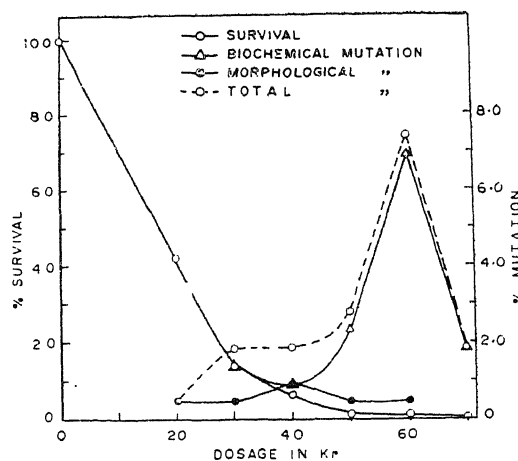


FIG. 1. Per cent survival of ascospores and per cent biochemical and morphological mutations in relation to gamma irradiation.

In the present investigation, however, loss of cellulolytic activity was observed in general with all the morphological mutants as could be found with those obtained by treatment with formaldehyde and hydrogen peroxide<sup>12</sup>. The biochemical mutants produced less cellulolytic enzyme as compared to the wild strain (Table II). Importance of biochemically deficient mutants of microorganisms in the study of metabolic pathways of organic

TABLE II  
Characterization of biochemical mutants of *Chaetomium aureum*<sup>a</sup>

| Sl. No. | Code No. with markers <sup>b</sup>  | Characteristics of mutants <sup>c</sup> |                        |   |
|---------|---|---|------------------------|---|
|         |   | Colour of mycelium                      | Growth (Dry Wt.) in mg | % Cellulase activity as compared to the wild type <sup>a</sup> i.e., 100% |
| 1.      | 3-igg paba <sub>1</sub> <sup>-</sup>  | light grass green                       | 212.42                 | 59.53   |
| 2.      | 7 pgg paba <sub>2</sub> <sup>-</sup>  | pale grass green                        | 214.20                 | 25.35   |
| 3.      | 9-w paba <sup>-</sup> /thi <sup>-</sup>   | white                                   | 209.32                 | 68.93   |
| 4.      | 12-bb bio <sup>-</sup>  | buff brown                              | 210.88                 | 43.16   |
| 5.      | 18-pf nic <sup>-</sup> /paga <sup>-</sup> /bio <sup>-</sup> /ca-pant <sup>-</sup> /pyri <sup>-</sup>  | pale flesh colour                       | 206.34                 | 53.66   |
| 6.      | 19-igg nic <sup>-</sup> /bio <sup>-</sup> /pyri <sup>-</sup> /ca-pant <sup>-</sup>  | light grass green                       | 212.46                 | 41.71   |
| 7.      | 20-w Ca-pant <sup>-</sup> /bio <sup>-</sup> /pyri <sup>-</sup> /nic <sup>-</sup> /fol <sup>-</sup> /thi <sup>-</sup> /paba <sup>-</sup> /inos <sup>-</sup>  | White                                   | 209.76                 | 67.23   |
| 8.      | 25-pcp Ca-pant <sup>-</sup> /pyri <sup>-</sup> /nic <sup>-</sup> /rib <sup>-</sup> /paba <sup>-</sup> /thi <sup>-</sup> /fol <sup>-</sup> /cho <sup>-</sup> | pale congo pink                         | 209.26                 | 40.12   |
| 9.      | 26-pgg lys <sup>-</sup>   | pale grass green                        | 218.10                 | 48.14   |
| 10.     | 27-igg leu <sub>1</sub> <sup>-</sup>  | light grass green                       | 208.16                 | 71.13   |
| 11.     | 28-bb leu <sub>2</sub> <sup>-</sup>   | buff brown                              | 214.64                 | 35.40   |
| 12.     | 29 bb tryp <sub>1</sub> <sup>-</sup>  | buff brown                              | 210.34                 | 50.39   |
| 13.     | 30 w tryp <sub>2</sub> <sup>-</sup>   | white                                   | 207.50                 | 65.37   |
| 14.     | 33-bb xan <sup>-</sup> /thy <sup>-</sup> /hypo <sup>-</sup>   | buff brown                              | 212.88                 | 25.39   |
| 15.     | 35-pgy guan <sup>-</sup> /ad <sup>-</sup>   | pale greenish yellow                    | 209.52                 | 40.12   |
| 16.     | 40 w xan <sup>-</sup> /hypo <sup>-</sup>  | white                                   | 212.35                 | 50.49   |
| 17.     | 41-igg guan <sup>-</sup> /ad <sup>-</sup> /leu <sup>-</sup>   | light grass green                       | 211.84                 | 40.39   |
| 18.     | 42-pgg xan <sup>-</sup> /tryp <sup>-</sup> /lys <sup>-</sup>  | pale grass green                        | 207.85                 | 56.70   |
| 19.     | 44-w bio <sup>-</sup> /lys <sup>-</sup>   | white                                   | 210.42                 | 40.39   |
| 20.     | 45-w paba <sup>-</sup> /thi <sup>-</sup> /leu <sup>-</sup>  | white                                   | 216.42                 | 38.12   |
| 21.     | 46-pcp paba <sup>-</sup> /fol <sup>-</sup>  | pale congo pink                         | 211.58                 | 29.78   |
| 22.     | 49-pgg paba <sup>-</sup> /inos <sup>-</sup>   | pale grass green                        | 205.94                 | 71.40   |

<sup>a</sup> : wild type *C. aureum* having amber yellow mycelia (210.40 mg dry wt.); <sup>b</sup> : symbols for nutritional requirements—paba<sup>-</sup> = *h*-aminobenzoic acid; thi<sup>-</sup> = thiamine; bio<sup>-</sup> = biotin; nic<sup>-</sup> = nicotinic acid; Ca-pant<sup>-</sup> = Ca-pantothenate; pyri<sup>-</sup> = pyridoxine; fol<sup>-</sup> = folic acid; inos<sup>-</sup> = inositol; rib<sup>-</sup> = riboflavin; cho<sup>-</sup> = choline; lys<sup>-</sup> = lysine; leu<sup>-</sup> = leucine; tryp<sup>-</sup> = tryptophan; xan<sup>-</sup> = xanthine; thy<sup>-</sup> = thymine; hypo<sup>-</sup> = hypoxanthine; guan<sup>-</sup> = guanine; ad<sup>-</sup> = adenine; <sup>c</sup> = in presence of optimum concentration of respective requiring chemical; / indicates alternative requirements.

compounds has been stressed upon by a number of workers. Alikhanian *et al.*<sup>14</sup> reported an increase of 50% in the antibiotic activity over the control in the mutants of *Streptomyces rimosus*. Nutritional dependence of auxotrophic strains was found to influence the alternation of antibiotic yield in *Penicillium chrysogenum* by Sermonti<sup>15</sup> and McDonald *et al.*<sup>16</sup> and in *S. antibioticus* by Polsinelli *et al.*<sup>17</sup>. Modification of physiological character through mutation of genes, as is evidenced in the present investigation, confirms the results obtained with *Streptomyces indicus* Chakrabarty where antibiotic activity in nutritihnal mutants against *Curvularia lunata* was found to be less as compared to the wild strain<sup>18,19</sup>.

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\* (Modified Czapek-Dox); K<sub>2</sub>HPO<sub>4</sub>—0.25 g; MgSO<sub>4</sub>·7H<sub>2</sub>O—0.50 g; NaNO<sub>3</sub>—2.0 g; KH<sub>2</sub>PO<sub>4</sub>—0.75 g; KCl—0.50 g; Glucose (Analar)—15.0 g; Distilled water (Double)—1000 ml; pH—6.5; Purified agar agar<sup>12</sup>—15.0 g.

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#### RELATIVE GROWTH IN SOME SPECIES OF MEMBRACIDAE (HOMOPTERA : INSECTA)

INFORMATION on the post-embryonic development of insects often forms an integral part of taxonomic studies and the nymphal stages of several species of membracids provide significant data for the proper diagnosis at the generic and specific levels. A definite relation appears to exist between the length of the body and that of the anal tube in the nymphal instars of all the membracid species investigated. An attempt has been made here to present the relative growth of the anal tube of the nymphal stages with a view to assessing its taxonomic importance.

Thirty-seven species representing the two sub-families, the *Oxyrhachinae* (Tribe *Oxyrhachini*) and the *Centrotinae* (Tribes *Leptocentrini*, *Centrotini*, *Gargarini* and *Coccosterphini*) were collected from various parts of South India<sup>4</sup> and reared in the laboratory for a study of the post-embryonic development. In the study of the relative growth,

the formula  $Y = bx^k$  (Huxley<sup>5</sup>) was used, and the growth ratio (K) and the initial growth index (b) were calculated using this formula, where Y is the length of the allometrically growing part (anal tube), X, the theoretical value of Y when X equals unity; K is the constant at which Y grows in relation to X.

It would be evident from Table I that the growth ratios in all species of *Leptocentrus* are greater than those in other genera (K ranging from 1.0303 to 1.1339), with the exception of *Otinotus* species of the *oneratus* group. *Leptocentrus bajulans*, *L. moringae* and *L. baulhiniae* are taxonomically closely related and their growth ratios and initial growth indices also show striking similarity. In all the species of *Leptocentrus*, the nymphs possess conspicuously long anal tubes and this accounts for a high value of K. The conspicuously lower growth ratio (K less than 1) for the species of *Otinotus* belonging to the *indicatus* group, justifies the division of the genus *Otinotus* into two natural groups. With regard to the genus *Telingana*, K and b of both the species are notably similar, suggesting a close relation between them. The tribe *Leptocentrini* including the genera *Leptocentrus*, *Otinotus* and *Telingana* appears to present a higher growth ratio in contrast to the other tribes *Gargarini*, *Centrotini* and *Coccosterphini*.

An analysis of K and b in the six species of *Gargara* reveals a very close similarity in the growth pattern of the anal tube (K less than 1), with the exception of *G. extrema*. The similarity of the initial growth index of *Parayasa maculosa* and *Gargara madrasensis* suggests a relationship between these two species. The taxonomic characters of both adult and nymphal instars of the genus *Parayasa* reveal a closer relation to *Coccosterphus* and both these genera are placed in the tribe *Coccosterphini*. The similarity of growth pattern in *Coccosterphini* and *Gargarini* supports the view put forward by Capener (1968) that the *Coccosterphini* should be merged with the *Gargarini*.

Of the five species of *Tricentrus*, the initial growth index of *T. albomaculatus* and *T. decornis* appears to be identical. A steeper growth line for *T. decornis* indicates a higher growth rate. *T. pilosus*, *T. congestus* and *T. purpureus* appear to have nearly similar values of b. It is noteworthy that the nymphal characters of these three species are strikingly similar. Of the three genera of *Coccosterphus*, the relative growth of *C. minutus* and *C. tuberculatus* appears to be similar, while *C. paludatus* differs from the other two species, the magnitude of difference in growth pattern, in terms of growth ratio and initial growth index being so great as to place this species in a different genus.

TABLE I

Growth pattern of anal tube in relation to length of body in membracid nymphs

| Species               |                              | Growth ratio (K) | Initial growth index (b) | r (between log X and log Y) | Significance of r -P- |
|-----------------------|------------------------------|------------------|--------------------------|-----------------------------|-----------------------|
| Tribe: Oxyrhachini    | <i>Oxyrhachis tarandus</i>   | 0.6509           | 0.2857                   | 0.4492                      | 0.1                   |
|                       | <i>O. rufescens</i>          | 0.7648           | 0.2839                   | 0.9976                      | 0.001                 |
|                       | <i>O. minusculus</i>         | 1.0020           | 0.1995                   | 0.9985                      | 0.001                 |
|                       | <i>O. uncatus</i>            | 0.7829           | 0.2095                   | 0.9858                      | 0.005                 |
|                       | <i>O. krusadiensis</i>       | 0.9826           | 0.1807                   | 0.8902                      | 0.05                  |
|                       | <i>O. brevicornutus</i>      | 0.8412           | 0.2320                   | 0.9948                      | 0.001                 |
| Tribe: Leptocentrini  | <i>Telingana nigroalata</i>  | 0.8900           | 0.2511                   | 0.9953                      | 0.001                 |
|                       | <i>T. consobrina</i>         | 0.8873           | 0.2396                   | 0.9912                      | 0.001                 |
|                       | <i>Leptocentrus taurus</i>   | 1.1267           | 0.2664                   | 0.9996                      | 0.001                 |
|                       | <i>L. rhizophagus</i>        | 1.2164           | 0.1905                   | 0.9730                      | 0.01                  |
|                       | <i>L. varicornis</i>         | 1.1339           | 0.2972                   | 0.9931                      | 0.001                 |
|                       | <i>L. leucaspis</i>          | 1.2288           | 0.2427                   | 0.9641                      | 0.001                 |
|                       | <i>L. moringas</i>           | 1.0982           | 0.3357                   | 0.9997                      | 0.001                 |
|                       | <i>L. bajulans</i>           | 1.0146           | 0.3558                   | 0.9993                      | 0.001                 |
|                       | <i>L. nigra</i>              | 1.1250           | 0.2855                   | 0.9957                      | 0.001                 |
|                       | <i>L. bauhiniae</i>          | 1.0353           | 0.3456                   | 0.9978                      | 0.001                 |
|                       | <i>L. mangiferae</i>         | 1.0383           | 0.3168                   | 0.9617                      | 0.01                  |
|                       | <i>L. major</i>              | 1.1206           | 0.2679                   | 0.9924                      | 0.01                  |
|                       | <i>Otinotus oneratus</i>     | 1.0735           | 0.1897                   | 0.9863                      | 0.005                 |
|                       | <i>O. mimicus</i>            | 1.2289           | 0.1673                   | 0.9764                      | 0.005                 |
|                       | <i>O. indicatus</i>          | 0.9328           | 0.2325                   | 0.9862                      | 0.005                 |
|                       | <i>O. obliquus</i>           | 0.9560           | 0.2675                   | 0.5755                      | 0.01                  |
| Tribe: Centrotrini    | <i>Tricentrus pilosus</i>    | 0.9051           | 0.2304                   | 0.9820                      | 0.005                 |
|                       | <i>T. albomaculatus</i>      | 0.7616           | 0.2511                   | 0.9936                      | 0.001                 |
|                       | <i>T. decornis</i>           | 0.8956           | 0.2557                   | 0.9955                      | 0.001                 |
|                       | <i>T. purpureus</i>          | 0.8973           | 0.2318                   | 0.9913                      | 0.001                 |
|                       | <i>T. congestus</i>          | 0.8514           | 0.2281                   | 0.9663                      | 0.01                  |
| Tribe: Gargarini      | <i>Gargara mixta</i>         | 0.7838           | 0.2370                   | 0.9363                      | 0.02                  |
|                       | <i>G. extrema</i>            | 1.0177           | 0.1969                   | 0.9994                      | 0.001                 |
|                       | <i>G. malabaricus</i>        | 0.8409           | 0.2383                   | 0.9921                      | 0.001                 |
|                       | <i>G. rustica</i>            | 0.8696           | 0.2463                   | 0.9935                      | 0.001                 |
|                       | <i>G. albitarsis</i>         | 0.8035           | 0.2342                   | 0.9983                      | 0.001                 |
|                       | <i>G. madrasensis</i>        | 0.8845           | 0.2249                   | 0.9941                      | 0.001                 |
| Tribe: Coccosterphini | <i>Parayasa maculosa</i>     | 0.9130           | 0.2265                   | 0.9831                      | 0.005                 |
|                       | <i>Coccosterphus minutus</i> | 0.8809           | 0.2069                   | 0.9949                      | 0.001                 |
|                       | <i>C. tuberculatus</i>       | 0.9419           | 0.2460                   | 0.9955                      | 0.001                 |
|                       | <i>C. paludatus</i>          | 0.5013           | 0.2881                   | 0.9392                      | 0.02                  |

As indicated in Table I, the growth ratio and initial growth index of *Oxyrhachis tarandus* and *O. rufescens* are very nearly similar except for the slightly higher growth rate in the first nymphal stage of *tarandus*. Taxonomically these two species appear very closely related. *O. uncatus* and

*O. krusadiensis* appear closely related and their initial growth indices also come closer.

In all the species discussed here, an inverse relation between growth ratio and initial growth index is evident. Matsuda<sup>3</sup>, in connection with the study of relative growth in Gerridae, put forward the

hypothesis "when the growth ratio increases, the initial growth index decreases and vice versa" which holds good for membracids also.

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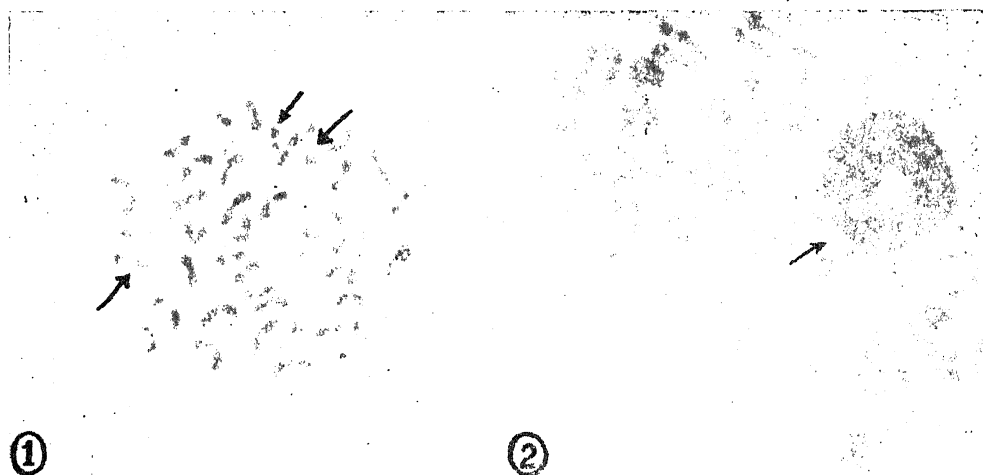
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# CENTROMERIC SENSITIVITY OF MOUSE CHROMOSOMES TO THE SYSTEMIC INSECTICIDE DIMETHOATE (ROGOR)

DIMETHOATE, which is an organophosphorus insecticide, is extremely poisonous to insects and mammals. It is physiologically a latent inhibitor of cholinesterase and other enzymes like trypsin and chymotrypsin and produces many pathological symptoms when internally

administered. The mutagenic effects of the insecticide DDT and its metabolites have been recently reported in *Drosophila*<sup>1</sup>. It is also known that Dimethoate undergoes degradation by the action of microsomes in liver cells<sup>2</sup>. Very recently it has been reported that insecticides like Dimecron and Rogor could produce a number of chromosomal anomalies in *Vicia faba* and *Gossypium barbadense*<sup>3,4</sup>.

Adults of both sexes of *Mus musculus* were injected with 1 c.c./100 g body weight of Dimethoate (0.5% and 1.0% solution). The control animals were injected with distilled water at the same rate. Standard cytological slides were prepared from the bone marrow cells of the animals sacrificed after 24, 48 and 72 hours by following colchicine-citrate-Giemsa-air drying technique. In the treated series, along with other aberrations, centromeric fission and stretching were predominantly seen. The frequency of centromeric fission was higher than centromeric stretching. The extent of chromosome affected with breakage at centromere ranged from 1 to 38 per cell (Fig 1). Instances of centromeric stretching in a good number of cells were also observed (Fig. 2). The frequency of both the types was highest at 24, moderate at 48 and least after 72 hours for the two doses (Table I).



FIGS. 1-2. Fig. 1. Metaphase plate with 3 centromeric fissions. Fig. 2. Metaphase plate showing centromeric stretching.

TABLE I

| Dose | Time (hrs) | Number of cells observed | Centromeric fission | Centromeric stretching | Total | Percentage |
|------|------------|--------------------------|---------------------|------------------------|-------|------------|
| 0.5% | 24         | 100                      | 51                  | 14                     | 65    | 65         |
|      | 48         | do.                      | 10                  | 7                      | 17    | 17         |
|      | 72         | do.                      | 8                   | 3                      | 11    | 11         |
| 1.0% | 24         | do.                      | 59                  | 11                     | 70    | 70         |
|      | 48         | do.                      | 39                  | 5                      | 44    | 44         |
|      | 72         | do.                      | 14                  | 2                      | 16    | 15         |

From the observed data, it is clear that the action of Dimethoate is specific to centromeric region and is of non-random type. Although the number of chromosome aberrations were produced in the bone marrow cells of Mongolian Gerbil, following the treatment with the herbicide, 2, 4, 5-T (2-4-5 Trichloro phenoxy acetic acid) no such localised aberration was reported<sup>5</sup>. In the case of meiotic chromosomes of grasshoppers<sup>6</sup>, the centromeric region did not show such type of sensitivity to Dimethoate. The aberrations reported here appear to have been produced by Dimethoate due to its direct action on the centromeric heterochromatin.

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#### RESPONSES OF THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI OF THE MUSK SHREW *SUNCUS MURINUS* L. TO HYPERTONIC SALINE ADMINISTRATION

It is generally accepted<sup>1-3</sup> that in mammals, the paraventricular nucleus (PVN) produces relatively more oxytocin than vasopressin (the antidiuretic hormone or ADH), while the supraoptic nucleus (SON) produces more vasopressin. Ablation of the PVN reduces the amount of oxytocin<sup>4-6</sup>. Moreover, in certain mammals<sup>6-8</sup> osmotic stimulation-induced changes are mainly observed in the neurosecretory cells of the SON. During an investigation<sup>9-13</sup> into the effects of drugs on the hypothalamic neurosecretory system of osmotically stressed musk shrews, it was noticed that the PVN responds to intraperitoneal (i.p.) hypertonic saline more than does the SON. The problem was, therefore, investigated in detail and the results are presented here.

Animals were divided into three groups, each comprising 50 adult individuals of either sex, and were treated as follows. *Group I*: Injection (i.p.) of 3% NaCl, 2 ml/animal/day, for 3 to 5 days. *Group II*: injection

(i.p.) of distilled water, 2 ml/animal/day, for 3 to 5 days. *Group III*: normal, untreated controls. Animals were killed by decapitation, the pituitary with adnexa was fixed in Bouin's fluid, embedded in paraffin, serially sectioned at 10  $\mu$  in the sagittal plane and stained with Gomori's aldehyde fuchsin and counterstained with light green and orange G.

The histology of the hypothalamic neurosecretory system of the musk shrew is described in detail elsewhere<sup>9-13</sup>. Distilled water administration (*Group II*) did not induce any alteration in the neurons of the PVN and SON. By contrast, administration of hypertonic saline induced remarkable changes in the neurons of the PVN (Fig. 1). The cell nuclei exhibited hypertrophy and the intranuclear inclusions were clumped into a compact mass, leaving a space between it and the nuclear membrane (Fig. 1). Marked depletion



FIGS. 1-2. Fig. 1. Portion of the PVN of a shrew treated with hypertonic saline (2 ml/day) for four days. Note the clumped intranuclear inclusions (arrows) and the depletion of NSM in the perikarya,  $\times 966$ . Fig. 2. Portion of the SON of the same animal. Note the normal appearance of the neurons and neuronal nuclei and heavy accumulation of NSM in the perikarya. cf. Fig. 1,  $\times 966$ .

neurosecretory material (NSM) from the perikarya of the neurons was also noticeable. In contrast to the neurons of the PVN, the neurons of the SON (Fig. 2) remained unaffected by hypertonic saline and were comparable to those of the controls (Groups II and III).

Our results indicate that in the musk shrew the PVN rather than the SON appears to respond to changes in the plasma osmotic pressure brought about by hypertonic saline administration. Since the responses of the hypothalamic neurosecretory cells to osmotic stress are presumably indicative of their augmented secretion of the ADH<sup>14</sup>, the PVN, rather than the SON, seems to be the major site of ADH production in the musk shrew. Hence the musk shrew appears to be different from other mammals<sup>1</sup> in which ADH production seems to predominate in the SON while the PVN produces more oxytocin. It may be noted that the PVN and SON in the musk shrew in their reaction to hypertonic saline administration resemble the PVN and SON of certain reptiles<sup>15-16</sup>.

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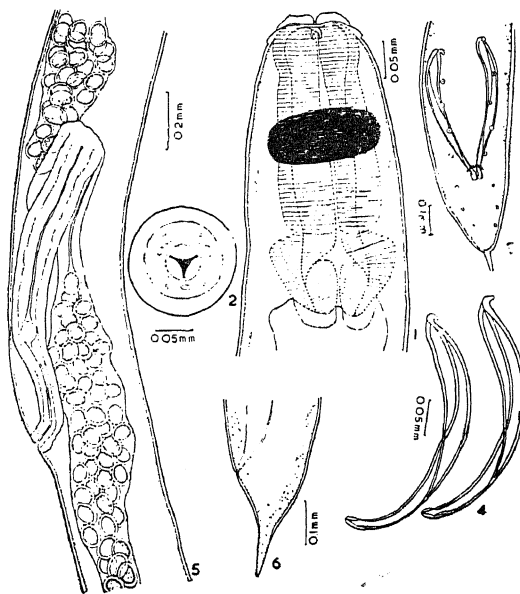
# ON A NEW AVIAN NEMATODE, *DUDEKEMIA CRISTATAI* SP. NOV. FROM A CRESTED LARK *GALERIDA CRISTATA* (LINNAEUS) FROM LUCKNOW

NUMEROUS specimens of the genus *Dudekemia* were collected from the intestine of a bird, Crested Lark, *Galerida cristata* (Linnaeus) from Lucknow. These specimens represent a new species and are designated *Dudekemia cristatai* sp. nov.

*Dudekemia cristatai* sp. nov. (Figs. 1-6)

## Description

Body slender (Fig. 1), medium sized. In end-on view (Fig. 2) mouth surrounded with three insignificant mobile lips and four cephalic papillae. Vestibule short. Pharynx triquetrous with cutting plates, irregular denticulate edges hinged at blunt corners. Oesophagus short muscular with a distinct pharyngeal swelling and distinct posterior bulb with three valves. Cuticle finely striated.



FIGS. 1-6. *Dudekemia cristatai* sp. nov. Fig. 1. Anterior end of male. Lateral view. Fig. 2. End-on view. Fig. 3. Posterior end of male. Ventral view. Fig. 4. Vulvar region. Lateral view. Fig. 5. Spicules. Fig. 6. Female tail. Lateral view.

**Male** : Tail short, conical. Eleven pairs of sessile papillae (Fig. 3) with four pairs of preanal and three pairs postanal. Spicules (Fig. 4) non-alate, similar and unequal. Gubernaculum absent.

**Female** : Tail short (Fig. 6), rounded, conical and subulate. Vulva post-equatorial with retrose, somewhat papilliforme anterior lip. Uterine branches



filled with numerous, large ellipsoidal eggs with thick smooth shell.

No form of this genus has been recorded so far from the vertebrate host. Eight species of the genus *Dudekemia* Artigas, 1930 have been described from millipedes, viz., *D. multipapillata* (Skr., 1916) Artigas, 1930; *D. brevicaudata* Artigas, 1930; *D. insularia* Ruiz et Coelho, 1955; *D. neyrai* (Singh, 1955 Trav. et Kloss, 1960 and *D. travassoi* Dollfus, 1964. The new form differs from all these species except *D. travassoi* in having spicules unequal instead of equal. The new form differs from it, in the arrangement of caudal papillae and in having specimens of larger size. In the new form there are 4 pairs preanal, 3 pairs postanal papillae, while in *D. travassoi*, 3 pairs preanal and 4 pairs postanal papillae. Accordingly, it is regarded as a new species with the specific name *Dudekemia cristatai* sp. nov.

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#### EFFECT OF MINERALS ON THE CHIASMA FREQUENCY IN DESYNAPTIC PEARL MILLET

CHROMOSOME pairing and chiasma formation during meiosis is under genetic control. These are affected by environmental factors, such as variation in temperature,

desynaptic stocks of rye and barley. Therefore, an attempt has been made to study the effect of phosphate and potash on the chiasma frequency in a desynaptic mutant of pearl millet, *Pennisetum typhoides* (Burn.) S. and H.

Selfed seeds from a desynaptic mutant were sown in the nursery beds. After five weeks, 15 plants in each treatment were transplanted to plots which were supplied with phosphate ( $P_2O_5$ ) at the rate of 93 kg per hectare and potash ( $K_2O$ ) at the rate of 25 kg per hectare. The control plot was not given any chemical. For cytological analysis, the young panicles were fixed in a 3:1 mixture of ethyl alcohol and acetic acid and squashes were made in acetocarmine. In each treatment 30 well spread MPCs were scored at MI for univalents, rod and ring for bivalents and the number of chiasmata per cell was worked out. The results were statistically analysed by using 't' test.

The results show that higher rates of phosphate and potash increased the number of ring bivalents and chiasmata per cell, while these treatments reduced the number of univalents and rod bivalents as compared to control (Table I). The number of ring bivalents increased from 1.60 to 3.00 and 2.77 per cell in the case of phosphate and potash treatments, respectively. The chiasma frequency increased from 5.43 in the control to 8.00 and 7.67 per cell in the treated plants. However, there was no significant difference between the treated samples.

The present study indicates that chiasma frequency can be increased by treating the desynaptic plants of pearl millet with phosphate and potash. In desynaptic plants, the genetic recombination is much less as compared to the normal plants. This recombination value may be increased by increasing the chiasma frequency after treatments with phosphate and potash.

TABLE I  
Chromosomal configurations and chiasma frequency after phosphate and potash treatments

|              | Univalents |     | Rad bivalents |      | Ring bivalents |      | Chiasma frequency |      |
|--------------|------------|-----|---------------|------|----------------|------|-------------------|------|
|              | Mean       | t   | Mean          | t    | Mean           | t    | Mean              | t    |
| 1. Phosphate | 3.40       | 1.6 | 2.23          | 0.74 | 3.00           | 0.74 | 8.30              | 1.46 |
| 2. Potash    | 4.20       |     | 2.13          |      | 2.77           |      | 7.67              |      |
|              | 4.10**     |     | 0.48          |      | 5.09**         |      | 5.21**            |      |
| 3. Control   | 6.35       |     | 2.30          |      | 1.60           |      | 5.43              |      |

\*\* Significant at 1% level; DF = 58.

ture<sup>1,6</sup>, chemical treatments<sup>2</sup>, and different types of radiations<sup>7,8</sup>. The mineral elements affect the chiasma frequency and some reports<sup>3-5</sup> show that higher rates of phosphate can increase the chiasma frequency in

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### CHROMOSOME NUMBERS IN THE GENUS *CEROPEGIA* LINN.

THOUGH Hooker<sup>1</sup> had enumerated 36 species of *CEROPEGIA* Linn., hardly 8 species have been recorded by Cooke and 10 species by Santapau and Irani<sup>2</sup> for the Bombay Presidency as against 21 species by Gamble<sup>4</sup> for peninsular India. Since Huber's<sup>5</sup> world monograph on the genus, eight new species have been described from the Sahyadri ranges alone by the junior author and other botanists from the Western Circle, Botanical

Survey of India. Except for three African species no cytological work has been done on this genus and the present study is the first report on eight Indian species of *CEROPEGIA*, three of which marked \* are newly described (Table I). Seven of the species studied are confined to the Western ghats of Maharashtra and only *C. BULBOSA* is commonly distributed throughout India. Somatic counts have been made from root tips after pretreatment in 8 oxyquinoline for 2 hours, fixing them in 1:3 acetic-alcohol and staining them in acetic-orcein following the normal squashing techniques. Voucher specimens are deposited in the regional herbarium of Botanical Survey of India (BSI), Poona and all the species studied are being grown in the experimental garden.

All the erect and twining species of *CEROPEGIA* studied reveal  $2n = 22$  only, suggesting a basic number of  $x = 11$ . Though Pardi<sup>6</sup> has reported  $2n = 44$  for both *C. debilis* N.E.Br. and *C. woodii* Schltr. [now treated under *C. LINEARIS* E. Mey. ssp. *DEBILIS* (N.E.Br.) Huber and ssp. *WOODII* (Schltr.) Huber respectively], no polyploids have been so far observed in any of the Indian species studied. The results with brief notes are summarised in Table I.

TABLE I

| Sl. No. | Name of species   | Voucher specimen               | Notes   |
|---------|---|--------------------------------|---|
| 1.      | <i>CEROPEGIA MACCANENSIS</i><br>Ans. nom. nov.<br><i>C. lawii</i> auct. non Hook. f.                | Sinhgad,<br>Ansari 97575       | Erect, common; leaves broadly ovate; flowers small. Confused with " <i>C. lawii</i> " in floras, the true <i>C. LAWII</i> Hook. f. recently collected from Harischandraghad, only.  |
| 2.*     | <i>C. SAHYADRICA</i> Ans. et Kulk.<br><i>C. panchganiensis</i> auct.<br>non Blatt. et McC.          | Ambolighat,<br>Kulkarni 108643 | Erect, confined to Ambolighat; leaves broadly ovate. Confused with <i>CEROPEGIA PANCHGANIENSIS</i> Blatt. et McC. which is restricted to Mahabaleshwar only.  |
| 3.      | <i>C. ATTENUATA</i> Hook. f.  | Kasara,<br>Billore 115319      | Erect; leaves linear. Allied to <i>C. MAHABALEI</i> Hem. et Ans. but distinguished by corolla lobes equal to or longer than tube.   |
| 4 a.    | <i>C. BULBOSA</i> Roxb. var.<br><i>BULBOSA</i>  | Junnar,<br>Hemadri 117930 A    | Twiner; leaves fleshy, ovate elliptic or orbicular.   |
| 4 b.    | <i>C. BULBOSA</i> Roxb. var.<br><i>LUSHII</i> (Grah.) Hook. f.                                      | Pashan,<br>Reddi 101214        | Twiner; leaves fleshy linear to linear lanceolate. Taxonomists have merged them together as extreme variants of one polymorphic species but in the absence of any intermediate forms and the seeds breeding true under cultivation, their varietal status is justified. |
| 5.      | <i>C. MEDIA</i> (Huber) A. s.<br>stat. nov.<br>( <i>C. evansii</i> McC. var.<br><i>media</i> Hube.) | Junnar,<br>Hemadri 117940      | Twiner. Huber's treatment of this species as a variety under <i>C. EVANSII</i> McC. is incorrect.   |

TABLE I (Contd.)

| Sl. No. | Name of species            | Voucher specimen               | Notes   |
|---------|----------------------------|--------------------------------|---|
| 6.*     | C. HUBERI Ans.             | Amba,<br>Ansari 105033         | Twiner; flowers white, small, showy, in flat topped heads. Restricted to Amba and nearby ghats. Rare and worthy of introduction in gardens. |
| 7.*     | C. SANTAPAU Wadhwa et Ans. | Mahad ghat,<br>Wadhwa 109651 A | Twiner. Infrequent; endemic to Satara District.   |
| 8.      | C. OCLATA Hook.            | Junnar,<br>Hemadri 117939      | Twiner. Common but restricted to Maharashtra.   |

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#### VARIATION IN DECAY RESISTANCE OF *BETULA PUBESCENS* EHRH. AGAINST *POLYPORUS VERSICOLOR*

THE problem of variation in decay resistance of wood, especially the commercial hardwoods of India, against wood rot fungi was investigated only by Bakshi (1962-67) and Bakshi *et al.* (1967). In view of the very little work done on this aspect, decay resistance of some hardwoods such as *Betula pubescens* Ehrh. (birch), *Azadirachta indica* A. Juss., and *Artocarpus heterophyllus* Lamk., against wood rot fungi *Polyporus versicolor* was worked out.

The present communication relates to the work on the variation in decay resistance of *Betula pubescens* against *Polyporus versicolor* L. ex. Fr. 651, a white rot fungus.

The material was obtained from *Betula* growing in the campus of Royal Holloway College, Englefield Green, Surrey, England. The tree was about 75 years old with a clear bole of 8.5 m long and a girth of 122 cm at breast height level. Three discs, one at 0.42 m,

second at 5.2 m, and the third at 8.36 m high from the base of the bole were cut and these were designated as D<sub>I</sub>, D<sub>II</sub>, and D<sub>III</sub> respectively in the present work. There was no differentiation of the wood into heartwood and sapwood. From each disc, planks of 2.5 cm thick were taken from one radius, passing through the pith. From each plank, strips of 2.5 cm thick were cut. From each of these, strips of 2.5 cm of wood next to the pith and the outermost region were removed and the experimental test blocks and the adjustment blocks of the size 2.5 cm × 2.5 cm × 0.9 cm (the smallest dimension being along the grain) were taken, one each from the inner region, middle region, and outer region.

Culture experiments determining the decay resistance of test blocks were conducted in 250 ml Erlenmeyer flasks by the soil block method (American Standard for Testing and Material D-2017-63 designation).

Feeder strips of the size 2.5 cm × 2.5 cm × 0.3 cm (the smallest dimension being across the grain) and reference blocks of similar size, as those of test pieces, were obtained from the wood of *Eriodendron*. Customary methods of sterilization were followed after which the flasks were inoculated with pure culture of *P. versicolor* three weeks before the commencement of test, during which period, the fungus attained a luxuriant growth on the feeder strip. Sterilized test blocks, and reference blocks were introduced into these flasks after determining their initial oven dry weight. Test blocks in triplicate from inner, middle and outer regions taken from each of the three different heights of the bole were kept in separate flasks. Simultaneously, one block, (the adjustment block) from each of these regions was maintained in the flasks which were not inoculated with the fungus in order to adjust the difference, in the final weight of the test blocks, brought about by factors other than the causative agent. The test was conducted for 16 weeks when 60-65% loss of weight occurred in the reference blocks. Final oven-dry weights of the adjustment blocks and the test blocks from which the surface mycelium was

removed were determined. Since the loss of weight in the adjustment blocks was less than 5%, it was not considered for adjustment as suggested by Bakshi (1962-67). The percentage loss of weights of the test blocks is depicted in Fig. 1, where each reading represents an average weight of three test blocks.

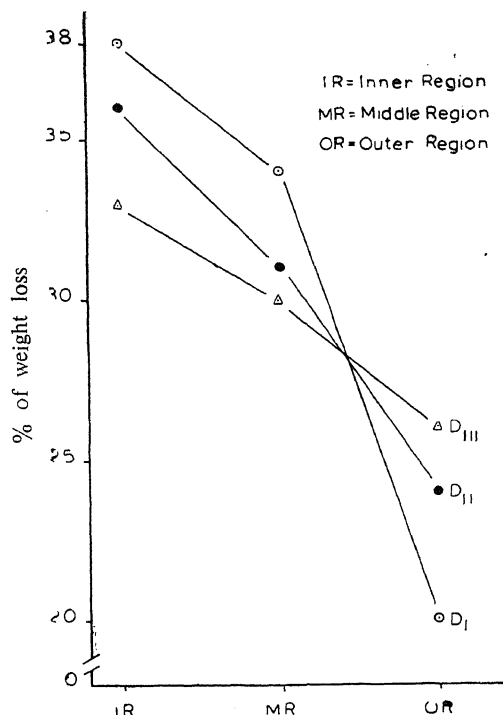


FIG. 1. Variation in decay resistance in *Betula pubescens*.

D<sub>I</sub> : Disc No. 1; D<sub>II</sub> : Disc No. 2; D<sub>III</sub> : Disc No. 3.

It is evident from the figure that there are two definite trends of variation in decay resistance in the bole of birch; (1) a progressive increase in decay resistance of the wood from inner to outer regions at all the three levels in the bole, and (2) an increase in decay resistance of the inner wood from base upwards while it is the reverse in the outer region of the wood. These observations are in conformity with those of earlier workers for various types of woods, Cartwright (1942) in larch Scheffer and Duncan (1947) in certain Central American and Ecuadorian woods, Scheffer and Hopp (1949) in *Robinia pseudoacacia*, Scheffer (1957) in Western red cedar, Rudman (1964) in *Eucalyptus* and Bakshi (1962-67) in some Indian woods.

Decay resistance of wood in general depends upon the quality and quantity of the extractives present in the wood (Cartwright and Findlay, 1958, Englerth and Scheffer, 1954). The amount of the extractive

deposited in the cells of wood is less in the juvenile stages of the growth of the tree. Quality and quantity of the extractives, however, progressively increase as the tree grows older. This fact may account for the type of vertical variations that have been observed in this investigation. The wood of inner region has been found less durable than that of middle and outer region and this can be explained by the gradual detoxification of the fungitoxic extractives due to polymerisation, acid hydrolysis and oxidation (Da Costa, 1973) or by the deterioration of the resistance factor with the advancement of age and continuous outward migration of previously formed resistance factor (Scheffer and Hopp, 1949).

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#### DIE-BACK IN CITRUS AND ITS RELATIONSHIP TO CERTAIN CHARACTERS

GENERALLY chlorosis and shoot growth have been used to express the relative degree of die-back. Certain other characters like chlorophyll concentrations, leaf area, leaf number, dry matter, nutrient composition of the leaves are also affected adversely in trees showing die-back. An attempt has been made to estimate the die-back of citrus trees by

studying partial regression coefficient of different characters.

In this study, investigations were conducted on chlorosis, chlorophyll, leaf number, shoot growth, dry matter, nitrogen and potassium of four trees each of Excelsior and Foster grape fruit, Kinnow mandarin, Ruby orange and Sampson Tangelo in March, June and September flushes during the year 1973 and 1974. The intensity of die-back of the individual trees of the different species used in this investigation were graded from 1 to 6 on the basis of visual observations.

Correlation coefficients between die-back and other characteristics and the regression coefficients of die-back on these characters were calculated by multiple regression technique<sup>2</sup>. Among the nutrients only nitrogen and potassium were included in the study as these were less than the optimum. The predictability of die-back with the help of different characters was estimated by calculating  $R^2$ , the coefficient of determination<sup>2</sup>. The correlation coefficients of die-back with all the eight characters were highly significant. However,  $R^2$  values indicated that only three characters, viz., nitrogen, chlorosis and chlorophyll were important in predicting the die-back, as their values, ranged from 66% to 94%. For other characters, the  $R^2$  values were comparatively low, their values ranging from 44 to 64%.

An attempt was also made to examine the predictability of die-back (as judged by  $R^2$ ) when nitrogen, chlorophyll and chlorosis were not available. The regression equations for different combinations of the remaining characters and their  $R^2$  values were calculated. The number of leaves when combined with shoot growth gave a  $R^2$  value of 55% which rose to 57% when shoot growth was replaced by leaf area. When all the three characters, leaf area, leaf number, and shoot growth, are taken into consideration for estimation, the  $R^2$  value rose to 66%. It is interesting to note that  $R^2$  values of these characters when taken individually ranged from 44% to 49% only.

It is clear from above discussion that die-back can be estimated efficiently either by taking into account characters like chlorosis, chlorophyll and nitrogen or by considering characters like leaf area, leaf number and shoot growth together. However it may be noted that while the maximum value of  $R^2$  was 94% in the first set of characters, it was only 66% in the second set of characters.

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### THE INFLUENCE OF SMUT ON THE PRODUCTIVITY OF *ISEILEMA LAXUM* HACK

*Iseilema laxum* Hack is a prominent and promising fodder grass<sup>1</sup> in Ujjain. In a survey of smut diseases of graminaceous plants, a majority of flowering stalks of *I. laxum* was found to be infected by a fungal disease. Preliminary observations showed that the causal organism was a smut, the *Sphacelotheca anayati*. The infected spikelets start appearing in the last week of September. Ovaries of the infected spikelets transform into oval dark brown sacs full of spores. The present study was undertaken to analyse the effects of smut disease on the primary productivity and the energy content of the host.

Healthy and infected plants were collected and analysed during late October to assess the above parameters. The disc method<sup>2,3</sup> was used to evaluate net primary production. The calorific values were determined with an oxygen bomb calorimeter, and the estimates were made by the formula proposed by Lieth<sup>4</sup>.

It is seen that the production rate of infected plants was reduced significantly (Table I). The reduction in the net primary productivity (NPP) of leaves may be due to the reduction in photosynthetic efficiency or due to increased breakdown due to high respiration.

TABLE I  
Productivity of healthy and infected leaf mg/h

|                              | Healthy | Infected | Significance level |
|------------------------------|---------|----------|--------------------|
| Respiration (R)              | 0.175   | 1.00     | 5%                 |
| Net primary production (NPP) | 2.445   | 0.610    | ..                 |
| Gross production (GP)        | 2.620   | 1.610    | 1%                 |
| GP = NPP + R                 |         |          |                    |

The reduction in gross production (GP) of infected plant was 61.5%. Further, from Table I, it is evident that there is a proportional increase in the respiration with a decrease in the net production in the infected leaves. It appears that the infected plant was photosynthetically inefficient and at the same time catabolically overactive, leading to the significant reduction in NPP and GP of the infected plants. Lower energy content of the diseased plant parts indicate the nature of the pathogen as a consumer (Table II).

This reduction was 74% in the stem, 55% in the leaf and 54% in inflorescence, which was highly significant.

TABLE II  
Energy content in cal/g ash free dry matter

| Plant part    | Healthy | Infected | Signi-<br>ficar ce<br>level |
|---------------|---------|----------|-----------------------------|
| Stem          | 3183    | 2383     | 5%                          |
| Leaf          | 3655    | 2011     | 5%                          |
| Inflorescence | 4915    | 2782     | 1%                          |

Thus the overall ecopathological effects of smut of *Isilema* grass reveal reduction in the rate of photosynthesis and lower energy status of host plant parts. However, these metabolic losses occurred without

any significant changes in the morphology of the host.

Our sincere thanks are due to Prof. L. P. Mall for providing necessary facilities for the work.

School of Studies in Botany,  
Vikram University, Ujjain,  
February 19, 1975.

Km. K. DAWAR.  
V. P. SINGH.

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## SHORT SCIENTIFIC NOTES

### Antifungal Activity of Some C<sub>28</sub> Steroidal Lactones

Withanolides<sup>1</sup> and physalins<sup>2</sup>, a new group of C<sub>28</sub> steroidal lactones from family Solanaceae have been found to possess anti-tumour, anti-inflammatory and antibacterial activity<sup>3</sup>. In continuation of our work on the antifungal activity of Withaferin A<sup>4</sup>, we now report on the activity of other related compounds with different substitution patterns to establish the structure-activity relationship.

The compounds\* (Withaferin A, Withanolide E, Withanicandrin, and Physalin B) dissolved in ethanol were incorporated in Czepek's agar medium to obtain different concentrations and the antifungal activity of the compounds was seen against *Aspergillus flavus*, *Epidermophyton floccosum*, and *Cladosporium herbarum*.

Withaferin A inhibited the growth of fungi in concentrations varying from 250 to 500 µg/ml whereas other compounds were inactive at concentrations of even 1 mg/ml.

The presence of 4 β-OH; 27-OH; 17 β side-chain; 5 β, 6 β-epoxide, as seen in Withaferin A, seems to be significant for the biological activity. Withanolide E lacks 4 β-OH, 27-OH and has 17 α-sidechain, whereas, Withanicandrin has 5 α-OH, 6 α-, 7 α-epoxide, both these compounds being inactive. It is interesting to observe that physalins are almost biologically inactive possibly due to their highly oxygenated nature.

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Varanasi 221 005, July 21, 1975.

P. D. SETHI.  
R. L. KHOSA.

\* Withaferin, A (4 β, 27-dihydroxy-5 β, 6 β-epoxy-1-oxo-22 R-witha-2, 24-dienolide);  
Withanolide E (14 α, 17 α, 20 α-trihydroxy-5 β, 6 β-epoxy-1-oxo-17 S, 20 S-22 R-witha-2, 24-dienolide);  
Withanicandrin (5 α-hydroxy-1, 12-dioxo-6 α, 7 α-epoxy-22 R-witha-2, 24-dienolide);  
Physalin B (22 R-14 α, 17-14 β, 26-diepoxy-13, 20, 22-trihydroxy-1, 15-dioxo-16 α, 24-cyclo-13, 14-seco ergosta-2, 5-diene-18, 27-dioic acid, 18-→20, 27-→22 dilactone).

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### Screening for Genetic Host Resistance Against the Bacterial Leaf Spot Disease in Tomato Incited by *Xanthomonas vesicatoria* (Doidge) Dowson\*

Bacterial leaf spot disease in tomato incited by *Xanthomonas vesicatoria* is not so very serious in India at present. Field observations have, however, revealed that the disease can occasionally flare up in epiphytotic proportions. Locating genetic resistance and breeding resistant varieties will be the most effective method of controlling this disease. Alexander and Lincoln (1942) reported *Lycopersicon peruvianum* Linn. to be the most valuable source of resistance in tomato against the disease. Avezdezhnova (1967) detected varietal differences

in the intensity of the disease though no variety was found to be entirely resistant. Information on varietal resistance to the disease being not available in the country, studies were carried out on these aspects at the Indian Agricultural Research Institute, New Delhi, during the period 1968–1970.

Eight hundred and forty germplasm lines of tomato belonging to the EC (Exotic Collection) and IC (Indigenous Collection) series were obtained from the plant Introduction Division of the IARI and these were screened for resistance.

Five seedlings of each line were raised in earthen pots 30 cm size and the plants were inoculated at the flowering stage by spraying the bacterial suspension obtained from a culture aged 24 hours with OD around 0.70–0.87 at 610 m $\mu$ . A rocker sprayer was used for inoculative application of the suspension. High humidity was maintained by spraying water on the plants at regular intervals. Observations on the reaction of each line was recorded after a period of two weeks. The severity of leaf and stem infection, extent of yellowing and defoliation were all taken into consideration for rating the lines into the various categories of resistance.

Of the 840 lines tested, none was found to have any absolute genetic resistance. The lines rated in the category 'slightly diseased' were as follows:

**EC Lines:** EC 1143, 2699, 2751, 2804, 3218, 4532, 5632, 6050, 6591, 7919, 8259, 8286, 8741, 9412, 12489, 12491, 16059, 16271, 16278, 16290, 17168, 21606, 26318, 27900, 31820, 35237, 35250, 35274, 35282, 37274, 37301 and 42663.

**IC Lines:** IC 6486 P 2, 6504 Pl, 13940 A, 16060 Pl.

The authors are grateful to the Head of the Division of Mycology and Plant Pathology, IARI, New Delhi-12, for the facilities provided for these studies.

Rice Research Station,  
Mannuthy, Trichur,  
Kerala, April 25, 1975.

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\* Part of the theses submitted by the senior author to the Post-Graduate School, IARI, New Delhi, in part fulfilment of the M.Sc. and Ph.D. degree programmes.

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#### Barite Mineralization Near Village Khairasian, Dist. Pauri, Garhwal, U.P.

A promising zone of barite occurrence has recently come to light, on the hill-slope, about half a km south-west of Khairasian (78° 44' 30" E : 29° 53' 20" N), located five km south-east of Satpuli (78° 42' 45" E : 29° 55' N) on the left bank of Eastern Nayar river. Garhwal Dist., U.P. The mineralized zone is associated with the lowest quartzite member of the Nagthat Formation near its contact with the underlying greenish grey phyllites of Chandpur Formation. A barite band varying in thickness from 1 to 1.5 m occurs conformably overlying the associated quartzite and phyllite which have a regional NW–SE strike with 50°–55° southwesterly dips. The continuity of barite along the strike has been traced for over 1/2 km. towards the north-east of Malethi.

The barite is coarse and is of grey, greyish white, white and buff colours. Coarse crystalline variety of barite is found as vein fillings along fractures and joints in the adjoining quartzites. Microscopic study has revealed strain effects like bent cleavages and twin lamellae and granulation along fractures. Chalcopyrite, galena, sphalerite and pyrite are present in small amounts, apart from irregular patches of secondary iron oxides.

As the known occurrence of barite in the Himalaya are mostly associated with carbonate suite of rocks, the association of barite mineralization with metaclastic sediments in the present area is of interest. Association of barite beds inter-layered with quartzites of Pre-Cambrian age has also been recently reported from Talya, Chitradurg District, Karnataka (Radhakrishna and Srinivasaiya, 1974).

The origin and economic viability of the barite occurrence near Khairasian is being investigated and shall be reported soon.

The authors are grateful to Prof. R. S. Mithal, Head of the Department of Geology and Geophysics, for extending facilities for the above study.

Department of Geology and Geophysics,  
University of Roorkee,  
Roorkee (U.P.), July 22, 1975.

J. K. GUPTA.

N. G. K. NAJR.

R. S. CHATURVEDI.

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### Black Rot' of Pineapple—A New Record from South India

Pineapple (*Ananas comosus* L.) does not suffer from any major fungal pathogens in the field, but after harvest, 'Black rot' or 'Fruit rot' is found to be the chief cause of deterioration of the stored fruit. The causal fungus has been identified as *Thielaviopsis paradoxa* van. Hon.

The infection generally starts at the cut end of the stem in the form of small, circular, water soaked spots which are very soft. With the advance of the disease the spots enlarge and coalesce forming a large black patch extending throughout the fruit. The inside tissue is also invaded by the fungus, becomes very soft, black, watery and emits a foul smell. Market surveys conducted during the past two years have shown that the total loss of fruits due to the rottage may be as high as 10–15%.

A critical perusal of literature indicates that this disease is a new record from South India. Chemical control trials conducted at this Institute have shown that dipping the fruits for five minutes after harvest in thiabendazole (1000 ppm) or benomyl (2000 ppm) would ensure good protection against this disease.

Thanks are due to Dr. G. S. Randhawa, Director and Dr. H. S. Sohi, Sr. Plant Pathologist, for their interest in this study.

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Indian Inst. of Hort. Research,  
255, Upper Palace Orchards,  
Bangalore-6, August 4, 1975.

T. S. SRIDHAR.

### *Ephelis oryzae* Syd. on Some Hosts New to India

Different species of *Ephelis* have been reported on different hosts in India<sup>1-4</sup>. During 1966 and 1974, *Ephelis* spp. was collected on some grasses (*Eragrostis tremula*\*, Hochst., *E. ciliaris* R. Br. var. *clarkei*\*, *Echinochloa colona*† Link, *Pennisetum alopecuroides*\* Steud., *Paspalum distichum*\* L.) and millets (*Paspalum scrobiculatum*† L. and *Setaria italica* Beauv.), at J.N.K.V.V. Farm, Jabalpur. Diseased samples of *Oryza sativa* L. from Surguja and of Bastar Districts also revealed the presence of this fungus. In all the cases, the causal organism transformed the panicles into a compact agarbatti-like shape.

On the basis of symptomatology and morphological characters, the present organism is identified as *Ephelis oryzae* Syd. The specimens have been

deposited in the Central Herbarium of Plant Pathology, J.N.K.V.V., Jabalpur, M.P.

Department of Plant Pathology & R. P. MISHRA.

Plant Breeding and Genetics, B. S. PALL.

J.N.K.V.V., Jabalpur, M.P., July 4, 1975.

\* New host records for the country.

† New host record for the State.

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### Record of *Plutella xylostella* Linn. [= *maculipennis* (Curt.)] as a New Pest of *Amaranthus viridis* in Karnataka

*Plutella xylostella* L. is an important pest of cruciferous crops attacking nearly 39 different host plants of the family<sup>3</sup>, apart from a number of weed hosts recorded<sup>2</sup>. Some of the non-cruciferous crops attacked are onion<sup>4</sup>, maize<sup>6</sup>, beet root, *Salsola kali* (Chenopodiaceae) and *Cirs arietinus* (Papilionaceae)<sup>5</sup> and okra<sup>1</sup>.

Recently (October–December, 1974), the caterpillars of *P. xylostella* were found along with the caterpillars of *Hymenia fascialis* on leafy vegetable, *Amaranthus viridis* in a few localised patches in Doddaballapur Taluk, Bangalore District. The nature of damage is similar to that met with on cruciferous crops. The present observation is the first record of an infestation of the pest on *Amaranthus viridis*.

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July 1, 1975.

M. VISHAKANTAIAH.

B. L. VISWESWARA GOWDA.

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## REVIEWS AND NOTICES OF BOOKS

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Proceedings of the Symposium on Physical and Dynamic Climatology held at Leningrad, USSR, in August 1971. (World Meteorological Organisation, Geneva), 1975. Pp. 398. Price : not given.

The subject of Physical and Dynamic Climatology was discussed in the Symposium under 6 sub-themes. There were one or more invited review-papers under each sub-theme and 30 contributed papers in all.

The discussion on the sub-theme *Energy Budget of the Earth* clearly brought out that radiation balance was one of the key-problems in Physical and Dynamic Climatology.

An important contribution on the sub-theme *Numerical Models of Climate* which is of special interest to us in India, is a mathematical model which, besides showing other changes between summer and winter, depicted well the monsoonal changes in India. This contribution as well as the other papers clearly brought out that the new physico-mathematical approach to the problem of climate has given an entirely different complexion to the subject of climatology as known hitherto. In fact, the participants in the symposium felt that a new discipline has emerged which could be named as *Climatology*.

The papers and discussions on *Satellite Climatology* revealed that although a vast amount of new material for climatological research has become available, there were important parameters like the total solar energy output which cannot still be continuously monitored by the Satellite technique.

One of the important papers on *The General Circulation of the Atmosphere* related to the development of a new model of tropical circulation incorporating higher and lower latitude effects.

The most exhaustive discussions during the symposium were on the topic *Climatic Fluctuations and Modifications*. Evidence was presented in papers, of man-made changes of climate and of the large-scale effects of carbon dioxide and aerosols on climate. There were also papers showing that over long geological intervals, tectonic movements and ocean-continent-atmosphere feedback mechanisms have profound influence.

A basic conclusion reached by the participants in the symposium was that the present state of knowledge was not advanced enough to lead to any plausible prediction of climate and that, therefore, there was imperative need to continue and intensify research in this field.

The proceedings of the symposium would be a valuable addition to any library containing publications on Atmospheric Sciences.

C. RAMASWAMY.

**Microbial Communities in a Forest—Rendzina Ecosystem—The Pattern of Microbial Communities.** By I. M. Szabo. (Academini Kiado, Publishing House of the Hungarian Academy of Sciences, Budapest), 1974. Pp. 376 + 408. Price \$ 25.00.

In recent times, the Microbial ecology of soils is receiving increasing attention because of the crucial role, the microbes play in the recycling of elements, the energy flow through the microbial community and the detoxication of pollutants. There is a great need for a thorough understanding of their activities to regulate the phenomena.

The book presents, the results of the elaborate and intensity studies of the author and his colleagues on the pattern of microbial communities in an extremely restricted ecosystem, the Forest-Rendzina. The author states that one of the purposes of the studies is "To recognise general principles which govern the activities of a complex saprophytic community in soil" but it is doubtful whether he has struck any new ground. Conceding the fact that there exists really no satisfactory method for classifying soil microorganisms, the author has not presented any new concept or idea in this regard. He has again mainly used taxonomic approach, using both conventional and computer techniques.

The versatility of the biochemical activities of the microorganisms and their extreme diversity have made it very difficult to correlate a particular type of soil with specific types of microorganisms as one could do with vegetation. Dr. Szabo writes "individual soil ecosystems have characteristic microbial communities of particular species combination which may in some degree repeat themselves in similar ecosystems"; but soon he comments that populations may be "functionally similar but differ in species composition". He gives his own observations on the four different sites investigated. Unfortunately the book does not give complete details of the soil parameters at the four different sites from where the samples were collected, to enable the reader to arrive at his own evaluation. At the same time, the author cannot be blamed for not correlating the results as there are many inherent difficulties in attempting such a correlation.

Notwithstanding the above, the book has plenty of refreshing information which a soil microbiologist will enjoy reading. The first chapter deals with the description of the ecosystem followed by a chapter on the pattern of growth of soil inhabiting microorganisms. He suggests classifying the "local events in soil as 'active', 'declining' and 'inactive'. In the next chapter, he brings out once again the crucial role of moisture and temperature in regulating soil biological phenomena. Chapter four deals with the actinomycete flora of the system. The observation that the larva of the insect St. Mark's fly (*Bibio marci*) dominates the soil invertebrate population is interesting. The author has also investigated the microflora of the gut of this larva and its role in litter decomposition.

In his studies on the rhizoplane microflora of black locust (*Robinia pseudoacacia*), the principal vegetation, he observes that the seedcoat microflora are not able to colonize the rhizosphere, as they are unable to stand the competition of the form, a finding which is significant.

It is in the last chapter that Dr. Szabo comes out with some of his beliefs. He is very forthright in refusing to accept Quastel's suggestion of treating soil as tissue in studying soil metabolism—an idea which is quite fashionable with many practicing soil microbiologists. He also disagrees with Kononova's ideas on humification processes.

While the book has several limitations and drawbacks, it is a valuable addition to any library dealing with microbial ecology".

V. N. VASANTHARAJAN.

**Arthropods as Final Hosts of Nematodes and Nematomorphs.** By M. R. N. Shephard. (Commonwealth Agri. Bureau, Farnham), Pp. 248. Price not given.

The author has made a valiant effort in bringing together the available bibliography on this topical subject and it should prove very useful to all the investigators in this research field. As the author himself has pointed out in the opening page of the book, it is very difficult to keep up to date with all the literature that is being published because of the vast research activity going on in the various scientific laboratories of the world. However, since this has been published in 1974, perhaps the author could have brought it up to date up to the end of 1973 instead 1972.

The bibliography is very well compiled and the brief abstracts presented with most of the references would help greatly the researcher in getting an idea about his requirements.

G. SWARUP.

**Water Plants of the World. : A Manual for the Identification of the General of Freshwater Macrophytes.** By Christopher D. K. Cook, Bernard, J. Gut, E. Martyn Rix, Jakob Schneller and Marta Seitz. (Dr. W. Junk, bv. Publishers, Hague), 1974. Pp. viii + 561. Price Dutch Glds. 120.

Water plants have received greater attention in recent years, largely because of their value as food for game animals, their tendency to foul and choke waterways, their association with mosquito breeding and their role in aquatic ecosystems. These plants, especially the underwater aquatics (e.g., *Echinodorus*, *Cryptocoryne*, *Vallisneria*, *Cabomba*) act as oxygenators to keep the water clean and healthy for the fishes. The present work is chiefly designed as a manual for the identification of freshwater macrophytes, but it also includes several semi-aquatic and marshland plants. The book describes c 470 genera belonging to the Charophyta (algae), Bryophyta (mosses and liverworts), Pteridophyta (ferns and fern-allies) and Spermatophyta (74 families). There are two general identification keys to the families and genera, one based on reproductive structures and the other on macroscopic vegetative characters. The families and genera are described in an alphabetical manner. Within the families, there are keys to the genera followed by their description and notes on the number of species, distribution, ecology, floral biology, systematic position and economic uses of noteworthy species. Taxonomic monographs and selected bibliographies are listed for several families and genera. The book is garnished with 426 line-drawings of carefully selected water plants depicting the habit, vegetative and floral parts, fruits and seeds. The illustrations of rare, interesting and unique species like *Regnellidium diphyllum* Lindm., *Ranalisma humile* Hutch., *Alternanthera philoxeroides* Griseb., *Cyperus papyrus* L., *Aldrovanda vesiculosa* L., *Hanguana malayanum* Merr., *Barclaya longifolia* Wall., *Ondinea purpurea* Hartog, *Victoria amazonica* Sowerby, *Trapella sinensis* Oliver, Podostemonads, Duckweeds, etc., add to the value and utility of the book. There is a useful glossary of technical terms, and a general index to family and generic names.

This informative and well-illustrated manual makes a pleasant reading that should prove useful to botanists, non-botanists and aquarists interested in the identification of common water plants, and in the use and management of aquatic ecosystems. There are at present few professionally written books on aquatic botany. This book with a taxonomic bias should serve as a valuable guide to the common fresh-water aquatics of the world.

J. K. MAHESHWARI.

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## OPTICAL ACTIVITY IN 1, 1'-BINAPHTHYLS

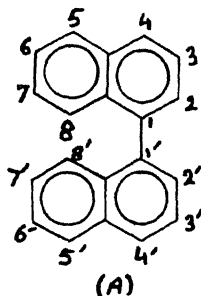
SHYAM SINGH

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### ABSTRACT

A general discussion of the optical activity and the optical stability of 1, 1'-binaphthyls is presented. Determination of the absolute configuration in such systems with the help of asymmetric synthesis, optical displacement rule and cotton effects associated with the shortwave band of the electronic absorption spectra is discussed.

AFTER the problem of the origin of optical activity in biphenyls<sup>1-3</sup> was solved, the same principles were applied to 1, 1'-binaphthyls (A)<sup>4</sup>. 1, 1'-Binaphthyls are 'atropisomers' in which under normal conditions the achievement of planarity becomes improbable; thus they possess optical activity. 1, 1'-Binaphthyl-2, 2'-dicarboxylic acid once resolved, did not racemise<sup>4</sup>. 1, 1'-Binaphthyl-8, 8'-dicarboxylic acid<sup>5-6,7</sup> was prepared in an optically active form; it exhibits a remarkably low optical



stability. The curious fact is that 1, 1'-binaphthyl-5, 5'-dicarboxylic acid<sup>8-11</sup>, which has no carboxylic acid group in the interfering positions (*e.g.*, 8, 8' and 2, 2') is optically more stable than 8, 8'-dicarboxylic acid. Another similar example, where the amino groups are not substituted in the interfering position of 1, 1'-binaphthyl, is 4, 4'-naphthidine<sup>12</sup>. The deamination of optically active 4, 4'-naphthidine at low temperatures yields optically active 1, 1'-binaphthyl (half life 13 min. at 50° in N, N-dimethyl formamide<sup>13,14</sup>). The spontaneous crystallisation of optically active 1, 1'-binaphthyl from its racemic melt<sup>15</sup> has been reported.

The degree of optical stability of chiral 1, 1'-binaphthyls depends mainly upon the effective size of the blocking barriers but the 8, 8'-dicarboxylic acid and its esters (flat groups) are exceptions. It was thus evident that an explanation of the low optical stability of 8, 8'-dicarboxylic acid, with two -COOH groups in blocking positions, must be sought outside the bounds of the simple obstacle theory of restricted rotation. A suggestion was advanced<sup>14</sup> that intramolecular overcrowding<sup>8</sup> in this acid

produces a state of strain which is relieved by distortions; these distortions are favourable to the occurrence of optical inversion by decreasing the barrier. On the basis of the studies on a set of 8, 8'-disubstituted compounds it was concluded that the energy of racemisation ( $E_{rac}$ ) is influenced by the following factors, *viz.*, steric barrier ( $E_{ster}$ ) to restricted rotation, the gain in resonance energy ( $E_r$ ) in the transition state, and the ground-state strain ( $E_{gs}$ ) of the molecule;  $E_{rac} = E_{ster} - E_r - E_{gs}$ . The entropy of activation and the conformation of the transition state also modify  $E_{rac}$ .

A large number of chiral compounds in the biphenyl series and some in the 2, 2'-positions of 1, 1'-binaphthyl series with a bridge running from one ring to the another have been reported during last 25 years. In such cases, the overlap of the substituents in the suitable positions is not the only principal cause of the restricted rotation<sup>1-3</sup>; the angular strain in the transition state of racemisation is also responsible. Before 1972, only the 2, 2'-bridged compounds were known as the optically active 1, 1'-binaphthyls; they are formed in such a manner that a ring larger than a five-membered one results. A six-membered bridged compound, 9, 10-dihydro-3, 4, 5, 6-dibenzophenanthrene<sup>16</sup> is optically active ( $E_{rac}$  30.8 kcal. mole<sup>-1</sup>). This is described as a dissymmetric nonasymmetric\* molecule. When the 2, 2'-positions are joined with a saturated chain resulting in a seven-membered ring, the compounds are optically more stable than those with six-membered rings<sup>16</sup>. The optical stability of the 2, 2'-bridged 1, 1'-binaphthyls cannot be compared with that of unbridged 1, 1'-binaphthyls, because the *trans*-passing route is not available. The low optical stability is in fact a dramatic change in the 2, 2'-bridged 1, 1'-binaphthyl series as the corresponding unbridged 2, 2'-disubstituted 1, 1'-binaphthyls are completely optically stable. The reason for this low stability in bridged compounds is that the passage through the planar transition state is

\* For an explanation of the significance of this term see Mislow, K., *Introduction to Stereochemistry*, Benjamin, Inc., New York, 1965, p. 27.

TABLE I  
Substituted 1, 1'-binaphthyls which racemise

| Sl. No. | Substituent at position |                      | $E_{\text{rac}}$<br>kcal.<br>mole <sup>-1</sup> | $\log_{10} A$ | $\Delta F \neq$<br>kcal.<br>mole <sup>-1</sup> | $\Delta H \neq$<br>kcal.<br>mole <sup>-1</sup> | $\Delta S \neq$<br>e.s.u. | Ref.   |
|---------|-------------------------|----------------------|---|---------------|--|--|---------------------------|--------|
|         | 8                       | 8'                   |   |               |  |  |                           |        |
| 1.      | H                       | H                    | 22.2  | 12.1          | 23.5   | 21.9   | -5.2                      | 14     |
| 2.      | H                       | COOH                 | 22.4  | 12.0          | 23.5   | 21.8   | -5.5                      | 14     |
| 3.      | COOH                    | COOH                 | 22.1  | 11.3          | 24.4   | 21.5   | -9.1                      | 14     |
| 4.      | COOMe                   | COOMe                | 22.0  | 11.6          | 23.8   | 21.4   | -7.5                      | 14     |
| 5.      | COOH                    | COOMe                | 21.6  | 11.4          | 23.7   | 20.9 (5)                                       | -8.4                      | 14     |
| 6.      | CH <sub>2</sub> OH      | CH <sub>2</sub> OH   | 29.2  | 12.6          | 29.8   | 28.4   | -3.4                      | 17     |
| 7.      | CH <sub>2</sub> OH      | COOMe                | 25.8  | 12.0          | 27.2   | 25.1   | -6.2                      | 17     |
| 8.      | CH <sub>3</sub>         | CH <sub>3</sub>      | 27.6  | 11.0          | 30.4   | 26.8   | -9.4                      | 17     |
| 9.      | COO <sup>-</sup>        | COO <sup>-</sup>     | 26.0  | 15.2          | 22.5   | 25.4   | -9.2                      | 14     |
| 10.     | COO <sup>-</sup>        | COOEt                | 25.7  | 14.0          | 24.0   | 25.1   | -3.2                      | 14     |
| 11.     | H                       | COOMe                | 23.6  | 12.5          | 24.1   | 22.9   | -3.5                      | 18     |
| 12.     | H                       | CH <sub>2</sub> OH   | 26.0  | 12.4          | 26.7   | 25.3   | -3.8                      | 18     |
| 13.     | H                       | CH <sub>3</sub>      | 25.3  | 11.7          | 27.2   | 24.6   | -7.3                      | 18     |
| 14.     | COOH                    | CH <sub>3</sub>      | 25.3  | 11.6          | 27.4   | 24.6   | -7.7                      | 20     |
| 15.     | CH <sub>2</sub> COOH    | CH <sub>2</sub> COOH | 32.0  | 14.3          | 29.6   | 31.2   | -3.6                      | 32     |
|         | 5                       | 5'                   |   |               |  |  |                           |        |
| 16.     | COOH                    | COOH                 | 24.1  | 12.3          | 25.4   | 23.5   | -5.9                      | 14, 19 |
| 17.     | COO <sup>-</sup>        | COO <sup>-</sup>     | 24.9  | 12.9          | 24.8   | 24.3   | -1.5                      | 14, 19 |
| 18.     | COOMe                   | COOMe                | 23.8  | 12.2          | 24.8   | 23.2   | -5.1                      | 14     |

mainly due to considerable reduction in the degree of interplanar angle in their ground state compared with the unbridged ones, thus the molecule requires lesser energy to pass through the transition state. On the basis of optical stability 1, 1'-binaphthyls can be divided into two categories:

1. Those which racemise (Table I).
2. Those which do not racemise (Table II).

TABLE II  
Substituted 1, 1'-binaphthyls which do not racemise

| Substituent at positions |                    |      |      |                   |                   | Ref.  |
|--------------------------|--------------------|------|------|-------------------|-------------------|-------|
| 2                        | 2'                 | 3    | 3'   | 5                 | 5'                |       |
| COOH                     | COOH               | H    | H    | H                 | H                 | 4, 16 |
| NH <sub>2</sub>          | NH <sub>2</sub>    | H    | H    | H                 | H                 | 21    |
| CH <sub>2</sub> OH       | CH <sub>2</sub> OH | H    | H    | H                 | H                 | 16    |
| SO <sub>3</sub> H        | SO <sub>3</sub> H  | H    | H    | H                 | H                 | 22    |
| OH                       | OH                 | COOH | COOH | H                 | H                 | 23    |
| NO <sub>2</sub>          | NO <sub>2</sub>    | H    | H    | SO <sub>3</sub> H | SO <sub>3</sub> H | 24    |

## STEREOCHEMICAL CORRELATIONS

Mainly the following methods have been applied to skewed biaryls (inherently dissymmetric) to determine absolute configuration.

(1) *Asymmetric Synthesis*.—In this method asymmetry is introduced in the course of a reaction, which involves the preferential formation of one or the other diastereoisomer in the reaction of *dl*-substance with an unsymmetrical reagent ( $k_L : k_d$ ) where  $k_L$  is the reaction rate constant for levorotatory isomer and  $k_d$  is the reaction rate constant for dextrorotatory isomer. The reagent approaches the part of the molecule to be reduced from the less hindered side. Meerwein-Ponndorf-Verley (M-P-V) reduction of a keto group has proved effective<sup>25</sup>. A binaphthyl ketone (IV) (Fig. 1) has been reduced<sup>26</sup>, by centrally asymmetric alcohols of known absolute configuration and resulted in configurational determination of biphenyls and later of 2, 2'-substituted 1, 1'-binaphthyls<sup>27</sup>. The stereospecificity of this method is generally applicable one. The ketone (IV) differs from ketones  $RR'C=O$  in the classical M-P-V reduction in two ways: (a) it can exist in enantiomeric forms, (b) hydrogen transfer to either face of the

carbonyl group in a given enantiomer produces the same alcohol. This is because, ketone (IV) is dissymmetric nonasymmetric. From inspecting models of the transition state, one expects that reduction of the R-isomer in S-octanol would take place quicker than that of the S-isomer. As S-(+)-2-octanol produces R-(—)-alcohol (V in Fig. 1) which is derived from (+)-1, 1'-binaphthyl-2, 2'-dicarboxylic acid (I in Fig. 1); this process relates the R-configuration for the corresponding optical isomers of the compounds in Fig. 1.

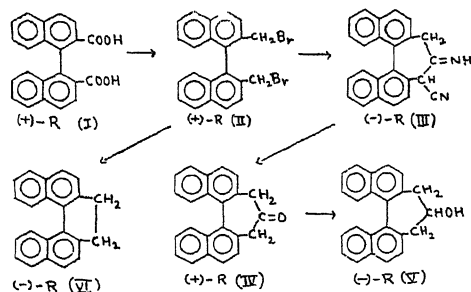


FIG. 1. Structures of some 2,2'-disubstituted 1, 1'-binaphthyls. The R-configuration has been assigned on the basis of asymmetric synthesis and chemical correlation<sup>27</sup>.

(2) *Optical Displacement*.—The success of Freudenberg's displacement rule in the centrally asymmetric chiral molecules has been discussed<sup>28</sup> in terms of the similarity in the size and the shape of the molecules to be compared. It is suggested that similar shifts in optical rotation take place in related derivatives of biaryls of the same configuration, and they make a basis for a chemical correlation. Using polarisability theory of optical rotatory power, the S-configuration has been assigned to (+)-9, 10-dihydro-3, 4, 5, 6-dibenzophenanthrene (VI). Therefore, its synthetic precursor, (—)-2, 2'-dicarboxylic acid (I), has also S-configuration. The absolute configurations of these compounds<sup>27</sup> confirm the validity of this rule. 2, 2'-Bridged compounds derived from S-(—)-6, 6'-dinitro-2, 2'-diphenic acid<sup>28</sup> and S-(—)-6, 6'-dichloro-2, 2'-diphenic acid<sup>28</sup> have stronger dextrorotatory power than their parent unbridged acids. 2, 2'-Bridged compounds derived from S-(+)-6, 6'-dimethyl-2, 2'-diphenic acid again show considerably larger dextrorotation. On the basis of these findings it has been proved<sup>28</sup> that going from unbridged to 2, 2'-bridged biaryls which involves a change in the interplanar angle, reflects a characteristic change in the sign and the magnitude of the optical rotation. It follows the general 'optical displacement rule' that

a symmetrically substituted hindered biaryl has the S- or the R-configuration, if in going from an unbridged to a bridged system, the optical activity suffers a marked shift in the positive or the negative direction respectively.

(3) *Chiroptical Effects*.—The detailed study of optical rotatory dispersion (ord) furnishes useful information about configurational assignment<sup>27</sup>. The usefulness of ord curves for such a purpose in optically active ketones of known absolute configuration has been tested. Circular dichroism (cd) of inherently dissymmetric chromophores (skewed biaryls) is extremely useful in separating the individual electronic transitions responsible for the total Cotton effect<sup>29</sup>. From the results of these two optical properties of chiral molecules it is concluded that the Cotton effect is related to the configuration of compounds. An enantiomeric pair of molecules have similar Cotton effects of opposite sign.

#### OPTICAL ROTATORY DISPERSION AND ABSOLUTE CONFIGURATION OF 1, 1'-BINAPHTHLYLS

Spectroscopic observations<sup>30</sup> have been collected for a series of unbridged and bridged biphenyls and unbridged and bridged 2, 2'-disubstituted 1, 1'-binaphthyls. Their configurations have been related to the compounds whose absolute configurations were already assigned by some other standard method. A change in conformation brings about a characteristic change in ord Cotton effect curve. The long-wave Cotton effect of the 2, 2'-bridged biaryls is generally accompanied by a Cotton effect at shorter wavelength, of opposite sign and of greater amplitude. The Cotton effect at the shorter wavelength usually dominates the sign of rotation in the visible region. For 2, 2'-bridged biaryls having the R-configuration, the sign of the long-wave Cotton effect is negative for 6, 6'-dinitro derivatives and positive for 6, 6'-dichloro and 6, 6'-dimethyl derivatives of biphenyl and 2, 2'-disubstituted 1, 1'-binaphthyls.

Unbridged 1, 1'-binaphthyls possess complex u.v. absorption spectra<sup>31</sup> dominated by maxima at about 285 and 230 nm. It corresponds to the complex ord spectra. Two ord Cotton effects for 1, 1'-binaphthyls with  $-\text{CH}_2\text{OH}$ ,  $-\text{CH}_2\text{Br}$ ,  $-\text{CH}_3$ ,  $-\text{COOH}$ ,  $-\text{COOMe}$ ,  $-\text{CONH}_2$  groups substituted in the 2, 2'-positions, centred at 285 nm and below 250 nm respectively, were observed<sup>30</sup>. In all the cases a positive or a negative 285 nm Cotton effect corresponds to the R- or the S-configuration respectively. A study of u.v. absorption spectra of 1, 1'-binaphthyl<sup>33</sup> and 8, 8'-disubstituted 1, 1'-binaphthyls<sup>32</sup> between 200–320 nm in ethanol has revealed more details. The ord spectrum of (+)-1, 1'-binaphthyl<sup>33</sup>



at 8° C in ethanol exhibits a negative Cotton effect centred at 285 nm confirming its S-configuration in conformity with X-ray crystallographic studies<sup>24</sup>. The ord spectra of twelve optically active 8, 8'-disubstituted 1, 1'-binaphthyls<sup>22</sup> exhibited in addition to a positive or a negative Cotton effect for R- or S-configuration respectively, a very strong negative or positive Cotton effect associated with the short wavelength u.v. absorption band.

Bridging with a saturated chain containing two carbon atoms incorporating the 2, 2'-positions of 1, 1'-binaphthyl changes bathochromically the u.v. absorption pattern compared with the unbridged compounds particularly at long wavelengths; bands at 320, 328, 335 and 348 nm appear<sup>25</sup>. Compound (VI in Fig. 1) shows a bathochromic shift of Cotton effect associated with its u.v. absorption shift; its Cotton effect is of enormous amplitude (1,570,000°) centred near 250 nm. In contrast, the ord curves of the bridged compounds, forming a seven-membered ring with the 2, 2'-positions, have u.v. absorption spectra dominated by maxima at 220, 232 and 306 nm. The bathochromic shift of the long wavelength absorption band at 283 and 293.5 nm of 1, 1'-binaphthyl<sup>23</sup> itself, in comparison with these bridged compounds also appears in ord curves yielding two Cotton effects of opposite sign, centred near 300 nm and below 240 nm respectively. A positive 300 nm Cotton effect corresponds to the R-configuration.

#### CIRCULAR DICHROISM AND ABSOLUTE CONFIGURATION OF 1, 1'-BINAPHTHLYLS

As pointed out earlier the cd curves are extremely useful in identifying the electronic transitions which are solely responsible for the individual Cotton effects. Intensity in the cd curves is analogous to extinction coefficient in the absorption spectrum and amplitude in the ord curve. All of them together characterise the internal chirality of chromophores present in a molecule. The sign of the cd curves corresponds to that of the related ord curve. The advantage of cd over ord is that the overlap of the tails in the bands of the latter is reduced in the former. It assists in the identification of weak optically active transitions generally not seen in ord. The cd studies have been undertaken in 2, 2'- and 8, 8'-disubstituted 1, 1'-binaphthyls<sup>20-22</sup>. Unbridged 2, 2'-disubstituted and 8, 8'-disubstituted 1, 1'-binaphthyls have a negative 285 nm Cotton effect corresponding to the S-configuration. All the 1, 1'-binaphthyls bridged in the 2, 2'-positions provide cd curves at 270 and 310 nm of opposite signs; they correspond to the oppositely signed ord curves centred near 265 and 300 nm respectively. Like in ord, a negative 300 nm cd Cotton effect corresponds to the S-configuration. Furthermore, a negative cd band at shortest wavelength (~220 nm) and a positive one 15-20 nm higher, is characteristic of S-configuration<sup>26</sup> (Table III).

TABLE III  
Correlation of configuration through cd in the short wavelength (214-240 nm) region<sup>22</sup>

| Substituents at positions | Sign of rotation at 589 nm | Negative extremum | Wavelength at $[\theta] = 0$ | Positive extremum | Configuration derived by cd |
|---------------------------|----------------------------|-------------------|------------------------------|-------------------|-----------------------------|
| S                         | S'                         |                   |                              |                   |                             |
| H                         | H                          | (+)               | 214*                         | 225*              | S                           |
|                           |                            | (-590,000)        |                              | (+825,000)        |                             |
| Me                        | Me                         | (+)               | 218*                         | 229.5*            | S                           |
|                           |                            | (-320,000)        |                              | (+1200,000)       |                             |
| COOH                      | COOH                       | (-)               | 223*                         | 240*              | S                           |
|                           |                            | (-5000,000)       |                              | (+420,000)        |                             |
| CH <sub>2</sub> Br        | CH <sub>2</sub> Br         | (-)               | 228*                         | 241*              | S                           |
|                           |                            | (-760,000)        |                              | (+142,000)        |                             |
| CH <sub>2</sub> COOH      | CH <sub>2</sub> COOH       | (+)               | 220*                         | 230*              | S                           |
|                           |                            | (-340,000)        |                              | (+135,000)        |                             |
| CH <sub>2</sub> (Py)-I-   | CH <sub>2</sub> (Py)-I-    | (+)               | 214*                         | 231*              | S                           |
|                           |                            | (-940,000)        |                              | (+280,000)        |                             |
| 2                         | 2'                         |                   |                              |                   |                             |
| Me                        | Me                         | (+)               | 221*                         | 228.5*            | S                           |
|                           |                            | (-590,000)        |                              | (+1240,000)       |                             |

\* Wavelength in nm.

Number in the parentheses represent the molar ellipticity  $[\theta]$ .

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## FRANCK-CONDON FACTORS FOR THE ELECTRONIC BANDS OF THE SECOND NEGATIVE SYSTEM OF O<sub>2</sub><sup>+</sup>

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### ABSTRACT

The Franck-Condon factors for the second negative system of O<sub>2</sub><sup>+</sup> is recalculated by the method suggested by T. Y. Wu. For the case of Morse Oscillators, an approximate method is suggested and tested by calculating the Franck-Condon factor for the (0, 0) band of the second negative system of <sup>16</sup>O<sub>2</sub>.

IN many problems, it is necessary to have a theoretical knowledge of the relative band strengths for the various bands in a band system for the estimation of the temperature of the emitting gas (Stellar atmosphere, aurora night sky) from the relative intensities of the bands of a molecule. These are usually approximated by Franck-Condon factors  $q_{v',v''}$ , the squares of the overlap integrals of the vibrational wavefunctions  $\psi_{v'}$

and  $\psi_{v''}$  of the upper and lower electronic states respectively,

$$q_{v',v''} = |I_{v',v''}|^2 = \left| \int_0^\infty \psi_{v'}(r) \psi_{v''}(r) dr \right|^2 \quad (1)$$

Equation (1) neglects a number of factors, including the dependence of the electronic transition moment on the internuclear separation  $r$  and rotation vibration interaction<sup>1</sup>.

For the evaluation of Franck-Condon factors, various approximate methods have been suggested<sup>2,3</sup>. Ta-Yu Wu<sup>4</sup> suggested a method that enables the overlap integrals to be evaluated semi-analytically and applied to the calculation of the Franck-Condon factors of the bands of second negative system of  $O_2^+$ . It can be easily seen from his results that the vibration sum rule<sup>5</sup> is not satisfied. Therefore, it is considered worthwhile to recalculate the Franck-Condon factors of the bands of the second negative system of  $^{16}O_2^+$  using the latest constants of Bhale and Narasimham<sup>6</sup>. The results of the present calculation are compared with those of T. Y. Wu as shown in Table I.

TABLE I

Franck-Condon factors of the bands of the second negative system of  $O_2^+$

| $v'v''$ | 0     | 1     | 2     | 3     | 4     | 5      |
|---------|-------|-------|-------|-------|-------|--------|
| 0 a     | 0.000 | 0.000 | 0.000 | 0.001 | 0.005 | 0.015  |
| b       | 0.000 | 0.006 | 0.450 | 2.210 | 8.020 | 87.500 |
| 1 a     | 0.000 | 0.000 | 0.001 | 0.006 | 0.020 | 0.047  |
| b       | 0.003 | 0.390 | 2.490 | 9.800 | ...   | ...    |
| 2 a     | 0.000 | 0.001 | 0.004 | 0.017 | 0.042 | 0.073  |
| b       | 0.120 | 2.270 | 7.300 | ...   | ...   | ...    |
| 3 a     | 0.000 | 0.002 | 0.010 | 0.031 | 0.061 | 0.074  |
| b       | 0.350 | 3.450 | ...   | ...   | ...   | ...    |
| 4 a     | 0.000 | 0.004 | 0.017 | 0.045 | 0.066 | 0.050  |
| b       | 0.810 | 6.960 | ...   | ...   | ...   | ...    |
| 5 a     | 0.001 | 0.007 | 0.026 | 0.054 | 0.056 | 0.021  |
| b       | 1.630 | 12.00 | ...   | ...   | ...   | ...    |

(a)  $q_{v',v''}$  by the method suggested by the T.Y. Wu of present calculation, (b)  $q_{v',v''}$  from the results of T. Y. Wu.

For many diatomic molecules, it is convenient and sufficient to represent the potential of a given electronic state by a Morse function. Using this Morse potential function<sup>7</sup> and the vibrational wavefunction<sup>8</sup> for the level  $v$ , the overlap integral  $I_{v',v''}$  can be expressed as

$$I_{v',v''} = (-1)^{v'-v''} \frac{N_{v'} N_{v''}}{a} \xi^{(k''-1)/2} \sum_{l=0}^{v'} \sum_{m=0}^{v''} (-1)^{l+m} B_l B_m I_{lm} \quad (2)$$

following the approach of Wu<sup>4</sup>.

Where

$$B_l = {}_v C_l (B' + v')_l, B_m = {}_{v''} C_m (B'' + v'')_m$$

$$(\delta)_l = \delta(\delta-1)\dots(\delta-l+1), (\delta)_0 = 1$$

$${}_v C_l = \frac{v!}{(v-l)! l!}$$

$${}_v C_m = \frac{v!}{(v-m)! m!} \xi^m \quad (3)$$

$$I_{lm} = \int_0^\infty \exp\left[-\frac{1}{2}(Z + \xi Z\gamma)\right] \quad (4)$$

$$P = \frac{1}{2}(k' + rk'') = 1 + \frac{1}{2}(1 + \gamma) + l - m\gamma \quad (5)$$

For  $a'$  is not equal to  $a''$ , the integral  $I_{lm}$  cannot be evaluated directly and therefore various substitutions have been tried to simplify the integral.

$$\text{Let } z/2 = y, \quad dz = 2dy \quad \text{and} \quad k = \xi/2\gamma + 1$$

Then  $I_{lm}$  can be transformed into

$$I_{lm} = 2^{p+1} \int_0^1 e^{-y(1+\xi\gamma)} y^p dy \quad (6)$$

By expanding  $e^{-k\gamma}$  and integrating<sup>9</sup>

$$I_{lm} = 2^{p+1} \Gamma(p+1) \sum_{r=0}^{\infty} \frac{(-k)^r \Gamma(p+1) \Gamma(r+1)}{\Gamma(p+1) \Gamma(r+1)} \quad (7)$$

For  $\Delta a$  close to 1, the above equation can be approximated as

$$I_{lm} = 2^{p+1} \Gamma(p+1) F(p+1, 1, 1, \dots, k) \quad (8)$$

where  $F(p+1, 1, 1, \dots, k)$  is the hypergeometric function.

The correctness of the above expression can be tested for  $a' = a''$  for which eq. (4) becomes

$$I_{lm} = \frac{2^{p+1} \Gamma(p+1)}{(\xi+1)^{p+1}} \quad (9)$$

For  $a' = a''$ , eq. (8) transforms into

$$I_{lm} = 2^{p+1} \Gamma(p+1) F(p+1, 1, 1, \dots, \xi) \quad (10)$$

For  $\xi < 1$ , the hypergeometric function in eq. (10) can be easily shown<sup>10</sup> to be  $(\xi+1)^{-(p+1)}$ . Therefore

$$I_{lm} = \frac{2^{p+1} \Gamma(p+1)}{(\xi+1)^{p+1}}$$

which is equivalent to eq. (9) implying the correctness of the procedure. Substitution of eq. (8) in to eq. (2) yields,

$$I_{v',v''} = (-1)^{v'-v''} \frac{N_{v'} N_{v''}}{a'} \xi^{(k''-1)/2} \sum_{l=0}^{v'} \sum_{m=0}^{v''} (-1)^{l+m} B_l B_m 2^{p+1} \Gamma(p+1) F(p+1, 1, 1, \dots, k)$$

from which Franck-Condon factors are calculated. For still higher accuracy, one has to use the eq. (7).

The method is applied for calculating the Franck-Condon factor ( $0.1 \times 10^{-5}$ ) for the (0, 0) band of second negative system of  $^{16}O_2^+$ . This negligibly small Franck-Condon factor obtained for the (0, 0) band is in harmony with the factor that the band is not experimentally observed.

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## ADSORPTION FROM *n*-HEXANE-BENZENE MIXTURES

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### ABSTRACT

Two samples of tin oxide, in the form of gel and precipitate have been prepared. Their surface properties like surface area, pore structure and surface acidity have been studied. Adsorption from *n*-hexane-benzene mixtures on these oxides reveals that *n*-hexane is preferentially adsorbed. This is explained in terms of low density of polar sites on the surface responsible for the adsorption of benzene molecule. It is observed that the surface of the tin oxide precipitate is more polar than the gel. In the case of tin oxide gel the porosity is also contributing to the preferential adsorption of *n*-hexane. Isotherms of concentration change are analysed by Schay-Nagy and Everett methods to yield the amounts of individual components in the adsorbed phase.

### INTRODUCTION

ADSORPTION from binary solutions of non-electrolytes has been used to characterise the nature of the surface including polarity, distribution and density of active sites and heterogeneity of the surface<sup>1-5</sup>. *n*-hexane-benzene is an interesting mixture for such investigations in view of the comparable areas but different geometrical disposition of the components. Kisilov and Pavlova<sup>6</sup>, from their adsorption studies on Linde molecular sieve 5A, using this liquid mixture, observed that *n*-hexane was completely taken up by the solid and benzene was excluded. They explained their result by assuming that the openings of the pores in Linde molecular sieve 5A were too small to admit molecules of benzene, but molecules of *n*-hexane were admitted in the vertical position. Zhdanov *et al.*<sup>7</sup> studied the adsorption of *n*-hexane-benzene mixtures on the zeolites and found that the interaction between the  $\pi$  electrons of the benzene ring and the ionic lattice of the zeolite was so strong that *n*-hexane was completely excluded over virtually the whole range of concentration. Exhaustive

investigations on a variety of solids is therefore necessary before we can know the circumstances favouring the adsorption of these components on the surface. The adsorption of *n*-hexane-benzene on tin oxide in the form of gel and precipitate is presented in this paper with a view to studying the influence of the structure of the adsorbent on the nature of adsorption. A study of this oxide system is of particular interest as the oxide is a semi-conductor<sup>9</sup> and an active catalyst for the oxidation of hydrocarbons<sup>8</sup>.

### MATERIALS AND METHODS

**Tin Oxide Gel.**—Equal volumes of 1.2 N ammonium hydroxide and 1.0 N stannic chloride solutions were mixed by vigorous shaking and the gel formed was allowed to settle overnight. The gel was washed several times with distilled water and then dried at 30° C and finally crushed to pass through a 100 mesh sieve.

**Tin Oxide ppt.**—Stannic oxide was prepared by the action of tin on con. nitric acid. The oxide was washed with distilled water to make it free from nitrate ions. It was dried at 120° C.

### Surface Area and Pore Structure

Surface area of the samples was determined by adsorption of nitrogen at -183° C using a volumetric apparatus. Pore structure was determined

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using a mercury porosimeter. The results are given in Table I.

TABLE I A

| Solid         | Surface area          | Surface acidity<br>meq/g | Pore vol.<br>cc/g | Porosity<br>cc/100 cc |
|---------------|-----------------------|--------------------------|-------------------|-----------------------|
| Tin oxide gel | 160 m <sup>2</sup> /g | 0.21                     | 0.18              | 50.5                  |
| Tin oxide ppt | 100 m <sup>2</sup> /g | 0.53                     | ..                | ..                    |

TABLE I B

Pore size distribution for the gel

| Pore size (Å) (diameter) | % by volume |
|--------------------------|-------------|
| 4-175                    | 72.1        |
| 175-300                  | 0.9         |
| 300-400                  | 0.0         |
| 400-500                  | 0.3         |
| 500-75000                | 26.7        |

The tin oxide ppt. is assumed to be non-porous and hence these data are not given.

**Surface Acidity.**—The samples (1 g) of the solid were shaken with 20 ml of *n*-butylamine solution of varying concentrations for 24 hours. After separating the solid, the filtrates were titrated with standard hydrochloric acid using bromocresol indicator. Blank solutions without the sample were also treated similarly. Difference in the titre values gave the surface acidity of the samples. These are given in Table I.

#### Adsorption Measurement

1 g of the solid was taken in each of the stoppered bottles along with 10 ml of *n*-hexane-benzene mixture of varying compositions and kept at 35° C for 48 hours, with occasional shaking. Blank solutions, without the solid were also treated similarly. Solutions were analysed refractometrically. Isotherms of concentration change were drawn as described by Kipling<sup>1</sup>.

#### RESULTS AND DISCUSSION

Table I gives the surface areas, surface acidity (*n*-butylamine adsorption value) of the two samples and the pore structure of the tin oxide gel. Figure 1 gives the composite isotherms, *i.e.*, plots of  $n_0 \Delta x_1 / m$  and  $x_1$  where  $\Delta x_1$  is the change in the mole fraction of *n*-hexane,  $n_0$  the moles of the benzene-*n*-hexane in contact with *m* g of the solids. It is seen that the isotherms for both the solids are U-shaped, *i.e.*, *n*-hexane is preferentially adsorbed on the oxide gel and the oxide ppt. over the entire concentration range. The solids have a heterogeneous surface with polar sites of the oxide. Because of the interaction of  $\pi$  electrons, the polar sites take up benzene while *n*-hexane is taken up by the non-polar sites. The larger non-polar surface is accommodating *n*-hexane, resulting in its higher adsorption. As the surface of tin oxide ppt. is more polar (Table I), benzene is adsorbed to a greater extent as compared with the oxide gel.

TABLE II

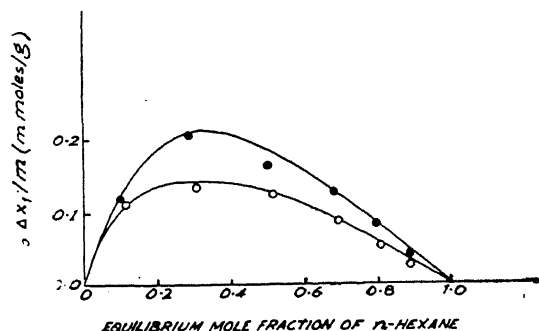
Adsorption from binary mixtures of *n*-hexane (1)–Benzene (2) at 35° C

| Adsorbent     | $x_1$ | $n_0 \Delta x_1 / m$<br>millimoles/g | $x_1 x_2$            | K <sub>a</sub> | $n^{\sigma}$ (Everett)<br>millimoles | $n^{\sigma}$ (Schay-Nagy)<br>Intercept method<br>millimoles |
|---------------|-------|--------------------------------------|----------------------|----------------|--------------------------------------|---|
|               |       |                                      | $n_0 \Delta x_1 / m$ |                |                                      |   |
| 1             | 2     | 3                                    | 4                    | 5              | 6                                    | 7   |
| Tin oxide gel | 0.1   | 0.11                                 | 0.82                 |                |                                      |   |
|               | 0.2   | 0.18                                 | 0.86                 |                |                                      |   |
|               | 0.3   | 0.20                                 | 1.05                 |                |                                      |   |
|               | 0.4   | 0.18                                 | 1.33                 | 4.75           | 0.53                                 | 0.44  |
|               | 0.6   | 0.15                                 | 1.60                 |                |                                      |   |
|               | 0.8   | 0.08                                 | 2.00                 |                |                                      |   |
|               | 0.9   | 0.04                                 | 2.25                 |                |                                      |   |
| Tin oxide ppt | 0.1   | 0.11                                 | 0.8                  |                |                                      |   |
|               | 0.2   | 0.14                                 | 1.1                  |                |                                      |   |
|               | 0.3   | 0.15                                 | 1.6                  | 6.50           | 0.36                                 | 0.28  |
|               | 0.4   | 0.14                                 | 1.7                  |                |                                      |   |
|               | 0.6   | 0.11                                 | 2.2                  |                |                                      |   |
|               | 0.8   | 0.06                                 | 2.7                  |                |                                      |   |
|               | 0.9   | 0.03                                 | 3.0                  |                |                                      |   |

TABLE III

Everett and Schay-Nagy analyses of adsorption from mixtures of *n*-hexane (1)-benzene (2) at 35° C

| Adsorbent     | $x_1$ | $x_1^\sigma$<br>(Everett) | No of millimoles in<br>the adsorbed layer<br>(Everett) |              | No. of millimoles in<br>the adsorbed layer<br>(Schay-Nagy) |              |
|---------------|-------|---------------------------|--|--------------|--|--------------|
|               |       |                           | $n_1^\sigma$   | $n_2^\sigma$ | $n_1^\sigma$   | $n_2^\sigma$ |
| Tin oxide gel | 0.1   | 0.35                      | 0.18   | 0.35         | 0.16   | 0.35         |
|               | 0.2   | 0.54                      | 0.28   | 0.25         | 0.28   | 0.20         |
|               | 0.3   | 0.66                      | 0.34   | 0.19         | 0.34   | 0.13         |
|               | 0.4   | 0.76                      | 0.40   | 0.13         | 0.36   | 0.10         |
|               | 0.6   | 0.87                      | 0.46   | 0.07         | 0.41   | 0.04         |
|               | 0.8   | 0.95                      | 0.50   | 0.03         | 0.43   | 0.01         |
|               | 0.9   | 0.98                      | 0.52   | 0.01         | 0.44   | 0.01         |
| Tin oxide ppt | 0.1   | 0.40                      | 0.22   | 0.14         | 0.14   | 0.18         |
|               | 0.2   | 0.60                      | 0.22   | 0.14         | 0.20   | 0.10         |
|               | 0.3   | 0.73                      | 0.26   | 0.10         | 0.23   | 0.06         |
|               | 0.4   | 0.82                      | 0.30   | 0.06         | 0.26   | 0.03         |
|               | 0.6   | 0.90                      | 0.32   | 0.04         | 0.28   | 0.01         |
|               | 0.8   | 0.96                      | 0.35   | 0.01         | 0.29   | 0.01         |
|               | 0.9   | 0.98                      | 0.35   | 0.01         | 0.29   | 0.001        |

FIG. 1. Composite isotherms for adsorption from *n*-hexane-benzene mixture. ● Tin oxide gel; ○ Tin oxide ppt.

In the case of tin oxide gel, the pore structure also seems to contribute to the preferential adsorption of *n*-hexane. About three-fourths of the pore volume of the gel is in the range 4–175 Å. As the pores having dimensions of nearly 5 Å can take up only *n*-hexane, preferential adsorption of this component is not difficult to explain. The maximum values of  $n_0 \Delta x_1/m$  are 0.2 and 0.14 m moles/g for the gel and the ppt, respectively. The adsorbed layer has been analysed by the models suggested by Schay-Nagy<sup>10-12</sup> and Everett<sup>13-14</sup>. According to Schay-Nagy, the linear portion of the curve when extended to  $x_1 = 0$  and  $x_1 = 1$  axes gives intercepts which correspond to  $n_1^\sigma$  and  $n_2^\sigma$ , i.e., the number of moles of components 1 and 2 in the

adsorbed phase in the concentration range where the isotherm is linear. This can lead us to the total number of moles in the adsorbed phase and then the isotherms can be analysed to give the individual adsorption values of the two components by means of the following equations:

$$n_0 \Delta x_1/m = n_1^\sigma x_1 - n_2^\sigma x_1 \quad (1)$$

$$\frac{n_1^\sigma}{(n_1^\sigma)_m} + \frac{n_2^\sigma}{(n_2^\sigma)_m} = t \quad (2)$$

$x_1$  and  $x_2$  are the mole fractions of *n*-hexane and benzene respectively in the liquid phase.  $n_1^\sigma$  and  $n_2^\sigma$  denote the number of individual components adsorbed  $(n_1^\sigma)_m$  and  $(n_2^\sigma)_m$  are the monolayer values of the solid for the two components. These are obtained by dividing the surface area of the solid by the molecular area of the adsorbate. Molecular areas in our case have been taken from the values reported in the literature<sup>15</sup> and  $t$  is the thickness of the adsorbed layer.

The other way of analysing the adsorbed phase, due to Everett, is given in terms of equations

$$\frac{x_1 x_2}{n_0 \Delta x_1/m} = \frac{m}{n^\sigma} \left( x_1 + \frac{1}{K_d - 1} \right) \quad (3)$$

$$n_0 \Delta x_1/m = n^\sigma (x_1^\sigma - x_1/m) \quad (4)$$

where  $K$  is the distribution constant, other notations have the same meaning as given earlier. The slope and the intercept of the plot of  $x_1 x_2 / (n_0 \Delta x_1/m)$  against  $x_1$  leads to the value of  $n^\sigma$  (the number of moles in the adsorbed layer) and  $K_d$  (the distribution constant).

Everett values of  $n^\sigma$  in the present investigation are 0.53 and 0.36 millimole for the gel and the ppt respectively. These values are in the ratio of the surface area of the solids. The monolayer values based on  $50 \text{ \AA}^2$  as the molecular area of  $n$ -hexane are 0.52 and 0.33 millimole. Thus the surface is completely covered during adsorption from this mixture. Possibility of perpendicular orientation of  $n$ -hexane is ruled out because  $50 \text{ \AA}^2$  is the molecular area in parallel orientation. Schay-Nagy values are slightly smaller. Table II gives the distribution constant  $K_a$  for the two solids. Individual adsorption of each component, calculated by the Schay-Nagy and Everett methods over the entire concentration range, is compared in Table III.

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## ON $A^{II}B^{IV}C_2^V$ TYPE MATERIALS

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### ABSTRACT

Attention is drawn to current developments in the materials technology of chalcopyrite type  $A^{II}B^{IV}C_2^V$  compounds. The problems associated with synthesis, characterisation, and crystal growth techniques are discussed in relation to the crystal structure. The tendency of selected compounds of this family to form ternary glasses which crystallise at relatively low temperatures is reviewed. Potential applications in the field of non-linear optics, switching devices, and glassy semi conductors are outlined.

### I. INTRODUCTION

THE development of a series of chemical compounds comprising elements of the II-IV-V groups has been at the forefront of technological "action" in the field of Material Science in recent years<sup>1,2</sup>. This interest has been stimulated by the possibility of several applications in areas such as glassy semiconductors<sup>3</sup>, non-linear optics<sup>4,5</sup> and growth of heterojunctions<sup>6</sup>. The successful use of these materials has been hampered, however, because of the lack of readily available high quality single crystals. Single crystals are necessary to perform experiments without the complicating and often uncertain effects of the grain boundaries. With the rapidly emerging potential of these materials, an intensive effort is underway in Materials Research Laboratories around the world to develop techniques of synthesis, characterisation, crystal growth and studies of glass-crystal transformations. It is the intent of this review to outline some of these developments.

### II. SYNTHESIS OF MATERIALS

(a) *Direct Fusion*.—The most commonly used method of synthesis is to seal the required amounts of the elements in evacuated quartz ampoules and heat at a relatively slow rate to one to two hundred degrees above the melting point of the compound; this prevents buildup of any high pressure volatile constituents (especially arsenic and phosphorus). To ensure homogenization of the melts, a vibratory rocking furnace may be used. For arsenides it may be necessary to apply a counter pressure of about 2 atmospheres of argon<sup>7</sup>. Following homogenization for several hours, the ampoule with contents is either cooled by shutting off the furnace or quenched directly in ice water. The synthesised materials are often crushed and remelted a number of times to ensure uniformity.

(b) *Glass Preparation*.—Although glass formation is well known in oxides<sup>8</sup> and chalcogenides<sup>9</sup>, the possibility of the compounds yielding glasses was first explored by Vaipolin *et al.*<sup>10</sup>. These workers

prepared glasses in the Cd-Ge-As system and reported properties of CdGeAs<sub>2</sub> glass and crystal materials. Other studies<sup>11</sup> have extended the glass forming range in the Cd-Ge-As system and attempts have been made to characterise the glass forming tendencies by thermal techniques<sup>12</sup>. CdGeAs<sub>2</sub> and CdGeP<sub>2</sub> are the only known members of the family of compounds that have been prepared as glasses; other compounds may also form glasses but have not been investigated.

(c) *Synthesis from Solutions*.—For compounds that tend to dissociate near the melting point, synthesis is often carried out by crystallizing solutions of the compound in molten metals. A desirable solvent is one which is also a part of the ternary compound. Commonly used solvents are tin and bismuth although indium and antimony have also been used. Many chalcopyrite type phosphides have been synthesised from tin solutions with fair degree of success but the arsenides often yield undesired phases. For example, attempts to crystallise CdGeAs<sub>2</sub> from tin solutions lead to the formation of germanium and a CdGe<sub>1-2</sub>Sn<sub>2</sub>As<sub>2</sub> phase<sup>13</sup>. As a general rule synthesis from solutions is very attractive for materials with a large temperature gap between the liquidus temperature and the peritectic point; the crystallisation of the solvent must occur below the peritectic.

(d) *Vapour Transport Synthesis*.—Vapour transport is generally used when the constituents have sufficiently high volatility. The transport process is carried out in sealed quartz ampules with a carrier gas (often iodine) in a controlled gradient furnace. The volatile constituents are swept from the hot end to the cold end of the furnace by the carrier gas and deposited under suitable conditions. Pre-synthesised material is often used in this method of synthesis although direct synthesis from elements of the ternary compound is also possible when the vapour pressure is sufficiently high (e.g., Zn, P). The vapour transport method has been successful used to synthesise ZnSiP<sub>2</sub><sup>14</sup>.

(e) *Sintering Techniques*.—This is a rarely used method for synthesis although it has special advantages in some cases. For compounds that have a high melting temperature it is often convenient to cold press mixed powders of the constituents and sinter the compact. BeSiN<sub>2</sub> is prepared by this technique by sintering the cold pressed pellets of the nitrides in an ammonia stream using a boron nitride crucible at temperature of 1750 to 1800°C.

### III. CRYSTAL STRUCTURE AND MATERIALS CHARACTERISATION

(a) *Crystal Structure*.—Crystallization of the II-IV-V<sub>2</sub> materials occurs in the crystal structure

derived from unit cells of wurtzite and sphalerite by the substitution of one type of atom in the cation sublattice of the initial structure by two different types of atoms. The resulting arrangement is of the "chalcopyrite" type and resembles two unit cells of zinc blende stacked on top of each other. The structure of a representative ternary compound of this family, ZnSnP<sub>2</sub>, is shown in Fig. 1, with the random arrangement of Zn and

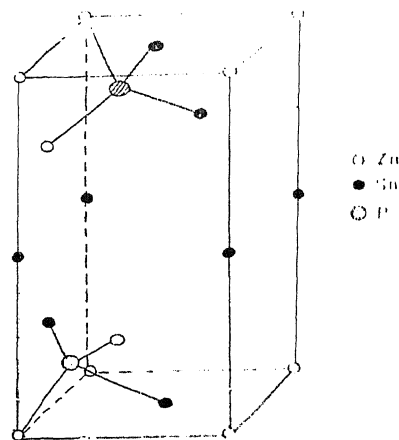


FIG. 1. Crystal Structure of chalcopyrites illustrated by the unit cell of ZnSnP<sub>2</sub>.

Sn atoms in the sublattice. The formation of the chalcopyrite structure by tetragonal compression of the sphalerite unit cell introduces a distortion in the lattice characterised by the quantity<sup>15</sup>,  $\sigma = a-c/a$ , where  $a$  and  $c$  are lattice constants. The larger the  $\sigma$  value the greater is the anisotropy in the thermal properties of the compound. The  $\sigma$  values for a number of compounds are listed in Table I.

TABLE I  
Tetragonal distortion value  $\sigma$  in some II-IV-V<sub>2</sub> compounds

| Material            | Lattice constant $a$ , Å | Lattice constant $c$ , Å | Tetragonal distortion $\sigma = 2c/a$ |
|---------------------|--------------------------|--------------------------|---------------------------------------|
| ZnSiP <sub>2</sub>  | 5.400                    | 10.441                   | 0.067                                 |
| ZnGeP <sub>2</sub>  | 5.465                    | 10.771                   | 0.040                                 |
| ZnSiAs <sub>2</sub> | 5.606                    | 10.890                   | 0.057                                 |
| CdSnP <sub>2</sub>  | 5.900                    | 11.518                   | 0.050                                 |
| CdSnAs <sub>2</sub> | 6.094                    | 11.918                   | 0.043                                 |
| CdSiP <sub>2</sub>  | 5.678                    | 10.431                   | 0.163                                 |
| CdSiAs <sub>2</sub> | 5.884                    | 10.882                   | 0.152                                 |
| CdGeP <sub>2</sub>  | 5.741                    | 10.775                   | 0.123                                 |
| CdGeAs <sub>2</sub> | 5.943                    | 11.2172                  | 0.112                                 |

(b) *Characterisation of Phases*.—The techniques used to characterise the phases obtained after



synthesis are X-ray diffraction, microscopy and differential thermal analysis (DTA). In some cases the more sophisticated methods of electron microscopy are used for structural studies and differential scanning calorimetry (DSC) for obtaining thermodynamic data. X-ray diffraction is almost always employed to ascertain the presence of crystalline peaks for the material being synthesised. The method is also useful for structure determination and lattice spacing studies. The DTA technique has been used with advantage in studying phase transformations. Being a quantitative technique (due to its dynamic nature) one can derive useful information on kinetics and thermodynamics of the material under observation. For instance, the DTA work on compounds like  $ZnGeP_2$  and  $CdSiP_2$  have successfully indicated the regions of glass formation and assisted in studies on order-disorder processes<sup>16</sup>. Crystallization activation energies for glass-crystal transformations have also been obtained by the DTA technique<sup>17</sup>. Optical microscopy with polarised light is a useful characterization tool specially to observe orientation differences in synthesised ingots. Imperfections can also be studied by this technique after suitable etching.

#### IV. SUMMARY OF CRYSTAL GROWTH TECHNIQUES USED

(a) *Growth from a Melt*.—The first reported attempt to grow single crystals from a melt was on the compound  $CdSnAs_2$ <sup>18</sup>. Material sealed in evacuated quartz ampoules was lowered at 2.5 mm/hour into a temperature gradient furnace. This standard method of crystal growth (Bridgman method) yielded polycrystalline ingots with a grain size of about 20 microns. In addition the top of the boules was often found to have two phases.  $ZnSnAs_2$  was grown<sup>19</sup> successfully as a single phase crystal by using graphitised quartz ampoules. Directional freezing in horizontal boats has also been attempted with some success for the compounds  $ZnSiAs_2$ ,  $ZnGeP_2$  and  $ZnSnAs_2$ <sup>20</sup>. In cases where the  $\sigma$  value (Table I), which represents the degree of tetragonal distortion in the chalcopyrite structure, is large the growth techniques from melts have very limited in success. For instance,  $ZnGeP_2$  ( $\sigma = 0.040$ ) was successfully grown from the melt<sup>21</sup> by the Bridgman technique but the same authors reported highly cracked crystal of  $CdGeAs_2$  ( $\sigma = 0.112$ ) when grown from a melt.

(b) *Growth from Solutions*.—The solution growth technique is more time consuming and requires careful temperature control (to prevent spurious nucleation) to get single crystal materials. The method has the following advantages :

- (i) since growth from solutions is carried out well below the normal melting points of the material being grown, no excessive pressure of the volatile constituents is built up ;
- (ii) high temperature phase transitions are avoided ;
- (iii) strain free crystals are obtained with fewer intrinsic defects.

The solution growth technique has been applied to grow a number of small single crystals (mainly phosphides) from tin solvents<sup>22</sup>. Recently  $CdGeAs_2$  has been grown from bismuth solutions in sizes upto 8–10 mm. Accelerated crucible rotation techniques have been suggested to improve quality and size<sup>23</sup>.

(c) *Vapour Growth*.—Growth from vapour transport normally yields small platelets with lengths upto 10 mm<sup>24,25</sup>. The method is unsuitable when large boules are desired.  $ZnSiP_2$  was grown by the vapour transport method<sup>26</sup> in an open flow system by mixing  $SiCl_4$ ,  $PH_3$  and zinc in hydrogen gas at 850° C. Vapour grown crystal often tend to be twinned with a capillary in the direction of the crystal axis. A clear understanding of the vapour mechanisms requires often the availability of enthalpies, dissociation free energies and other thermodynamic data. Such an attempt was made for a series of chalcopyrites<sup>27</sup> and the crystal growth was analysed.

In a brief review of the kind presented here it is not possible to list all the growth attempts used. The voluminous literature on the subject is summarised in Ref. 28.

#### V. PROPERTIES AND APPLICATIONS

The ultimate success of the compounds of the II–IV–V<sub>2</sub> will lie in the application of these materials in technological practice. It is gratifying to note that several applications are already known for this family of materials. In Table II are listed the physical properties of a few chalcopyrites and the specific applications reported so far are briefly outlined below.

Applications in infrared (conversion from the 8–12 micron range to below 1 micron) have been suggested for these materials<sup>21</sup> which makes them useful in advanced imaging detectors. Laser action has been reported in  $CdSnP_2$  at a wavelength of 1.01 microns when pumped by an electron beam<sup>29</sup>. The band gap of this material lies close to the 1.06 micron Nd:YAG laser emission thus making it a potential material for room temperature detectors. In addition possible applications have also been suggested<sup>30</sup> in optical devices such as limiters and passive Q-switches. The chalcopyrites have

TABLE II

Physical properties of a few chalcopyrites

| Material            | c/a ratio | M.Pt.<br>°C  | Phase<br>transition<br>temperature<br>°C | Energy<br>bandgap,<br>ev |
|---------------------|-----------|--------------|--|--------------------------|
| ZnSiP <sub>2</sub>  | 1.934     | 1370         | ..                                       | 1.99<br>2.30             |
| ZnGeP <sub>2</sub>  | 1.970     | 1020         | 952                                      | 2.0<br>2.4               |
| ZnSiAs <sub>2</sub> | 1.943     | 1038<br>1096 | ..                                       | 1.64<br>1.76<br>2.10     |
| CdGeAs <sub>2</sub> | 1.888     | 670          | 630                                      | 0.53                     |

also been recommended as materials in high frequency oscillators<sup>31</sup>.

#### VI. CONCLUDING REMARKS AND SUGGESTIONS FOR FUTURE WORK

The applications for the present materials open many challenging areas of research. From a processing point of view, the most urgent problem, perhaps, is development of phase equilibria data on the complex ternary compounds. Such phase studies shall be very useful in applying solution-growth techniques of crystal growth and will undoubtedly answer questions related to the existence of high temperature (metastable) phases. In the area of glassy materials, there is dearth of knowledge on crystallization kinetics<sup>32</sup>, morphology of crystals, and the energetics of the glass-crystal transitions. In addition potential exists for investigating and extending the glass formation areas in the family of II-IV-V<sub>2</sub> compounds. The role of phase separation or unmixing of the glasses in promoting or inhibiting the nucleation and growth kinetics also need exploration.

Much data have been reported on the electronic and physical properties of these materials. The non-availability of single crystals of sufficient size and quality is still a major hurdle, in the work on crystal physics. Many fascinating advances in the study and applications of these materials can be expected to be investigated, as the "art" of the crystal growing continues to be refined by the systematic studies of the Materials Scientist.

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My interest in materials has been initiated and sustained during educational years at I.I.T., Bombay and the University of California, Berkeley. I am also indebted to my former colleagues at the Center for Materials Research, Stanford University, where I first had the privilege of working on materials discussed in this review.

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## LETTERS TO THE EDITOR

CRYSTAL DATA ON MORPHOLINIUM  
METAVANADATE

MORPHOLINE is a base of moderate strength and can get easily protonated in acid medium to give bulky non-spherical morpholinium cation,  $[\text{morphH}]^+$ . As a part of general investigation on the stabilization of complex anions by bulky counter cations, in this note is reported the isolation and characterization by elemental analysis, X-ray diffraction and infrared spectral measurements of morpholinium metavanadate,  $(\text{morphH})\text{VO}_3$ .

The title compound was prepared by dissolving about 4 g of  $\text{V}_2\text{O}_5$  in about 20 ml 40% hot aqueous morpholine. The resultant solution was filtered and kept aside for 1 to 2 days. The colourless crystalline substance which separated out was filtered, washed with acetone and air-dried. Analysis: Found, C 25.9, H 5.50, N 7.57; V 26.7%; required for  $\text{C}_4\text{H}_{10}\text{NO}_4\text{V}$ , C 25.7, H 5.38, N 7.49, V 27.2%.

The X-ray powder diffraction patterns were taken on a Philips PW 1041 diffractometer using  $\text{CuK}_\alpha$  radiation. The incident angle,  $2\theta$ , was scanned from 5 to  $70^\circ$ . All the lines observed in the diffraction pattern were indexed for the cubic system satisfactorily and the unit cell dimension was found to be 9.881 Å. The observed and the calculated  $d_{hkl}$  values together with the relative intensities are given in Table I. The density of the crystals measured pycnometrically is  $1.23 \text{ g cm}^{-3}$ , while that calculated for 4 formula units of  $(\text{morphH})\text{VO}_3$ , from the unit cell constant is  $1.29 \text{ g cm}^{-3}$ . Since there were no systematic absences, the probable space groups<sup>1</sup> are P 23, Pm3, P432, P43m, or Pm3m.

The infrared spectrum of the compound measured on Perkin Elmer 225 spectrophotometer using CsI pellet technique showed characteristic V—O stretching frequencies ( $\text{cm}^{-1}$ ) at 934 s, 905 s, 868 s, 762 s, 635 s, 538 m, 338 m and 310 w. These frequencies are chosen by screening out the absorptions due to morpholinium moiety by comparing the spectrum with that of morpholinium sulphate. The values resemble the V—O vibrational frequencies<sup>2,3</sup> of  $\text{NH}_4\text{VO}_3$ , suggesting the V—O network in both the vanadates may be similar though ammonium metavanadate belongs to the orthorhombic crystal system<sup>4</sup>.

This work was carried out in W. Germany during the author's stay there and he is grateful to the Director, Anorganisch-Chemisches Institut Goettingen, for providing the necessary facilities.

TABLE I  
*X-ray diffraction data on morpholinium  
metavanadate*

| Sl. No. | $d_{hkl}$ (obs.) (Å) | $d_{hkl}$ (calc.) (Å) | Assignment (hkl) | Relative intensity ( $I/I_0$ ) |
|---------|----------------------|-----------------------|------------------|--------------------------------|
| 1       | 9.870                | 9.881                 | 100              | vs                             |
| 2       | 7.019                | 6.984                 | 110              | m                              |
| 3       | 5.712                | 5.704                 | 111              | m                              |
| 4       | 4.937                | 4.940                 | 200              | w                              |
| 5       | 4.425                | 4.420                 | 210              | vs                             |
| 6       | 4.037                | 4.034                 | 211              | vs                             |
| 7       | 3.490                | 3.493                 | 220              | s                              |
| 8       | 3.297                | 3.294                 | [300]<br>[221]   | s                              |
| 9       | 3.129                | 3.120                 | 310              | vs                             |
| 10      | 2.976                | 2.975                 | 311              | w                              |
| 11      | 2.851                | 2.853                 | 222              | m                              |
| 12      | 2.744                | 2.741                 | 320              | w                              |
| 13      | 2.635                | 2.640                 | 321              | vw                             |
| 14      | 2.473                | 2.470                 | 400              | m                              |
| 15      | 2.398                | 2.396                 | [410]<br>[322]   | m                              |
| 16      | 2.333                | 2.329                 | [411]<br>[330]   | w                              |
| 17      | 2.263                | 2.266                 | 331              | vw                             |
| 18      | 2.210                | 2.210                 | 420              | w                              |
| 19      | 2.169                | 2.157                 | 421              | vw                             |
| 20      | 2.106                | 2.107                 | 332              | w                              |
| 21      | 2.013                | 2.017                 | 422              | vw                             |
| 22      | 1.975                | 1.976                 | [500]<br>[430]   | m                              |
| 23      | 1.940                | 1.937                 | [510]<br>[431]   | w                              |
| 24      | 1.905                | 1.902                 | [511]<br>[333]   | w                              |
| 25      | 1.838                | 1.835                 | [520]<br>[432]   | m                              |
| 26      | 1.788                | 1.804                 | 521              | vw                             |
| 27      | 1.748                | 1.748                 | 440              | w                              |
| 28      | 1.720                | 1.719                 | [522]<br>[441]   | w                              |

$a_0 = 9.881 \text{ Å}$ ; s = strong, m = medium,  
w = weak; v = very.

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### STUDIES IN MIXED LIQUID CRYSTALS: RELIABILITY OF EXTRAPOLATION METHOD

CERTAIN chemical compounds having structures apparently favourable for forming liquid crystals do not exhibit mesomorphism on account of their melting points being too high. Such compounds may possess latent transition temperatures which may be experimentally determined. Use of the extrapolation of the transition curves for determining the latent transition temperatures was made by Bogojawlensky<sup>1</sup> and Walter<sup>2</sup>, but later on serious doubts<sup>3</sup> about its reliability were raised. To some extent our efforts<sup>4</sup> to repel the doubts have been fruitful. Continuing<sup>4</sup> our previous work, we have now studied the same ten Schiff's bases in mixtures with other liquid crystalline compounds, viz., *p*-propionoxybenzal-*p*-anisidine (series III) and *p*-propionoxybenzal-*p*-phenetidine (series IV) which are themselves Schiff's bases exhibiting nematic mesophase. Thus both the compounds of these binary systems have the same structure, the only difference being the polar terminal groups. The thermal ranges over which these mesomorphs show nematic property are different from those of *p*-azoxyanisole and *p*-azoxyphenetole. On smooth extrapolation of the transition curves of series III and series IV, the values of the latent transition temperatures for the various Schiff's bases are obtained, which are not only mutually comparable but are also almost concurrent with those obtained earlier<sup>4</sup>, as is evident from Table I.

Thus, we have further evidence which lends support to the reliability of extrapolation method. A detailed discussion and a complete report of the work under investigation will be communicated in due course. One of us (G.H.P.) thanks the Government of Gujarat, for financial support, under the SIRC Scheme, during the course of this study. Our thanks are also due to Professor S. M. Sethna for encouragement and help.

TABLE I  
Extrapolated Values of Latent Transition Temperatures  
(LTT) in °C

| Schiff's base as<br>component B                              | Series III<br>with compo-<br>nent A as<br><i>p</i> -propion-<br>oxybenzal-<br><i>p</i> -anisidine | Series IV<br>with compo-<br>nent A as<br><i>p</i> -propion-<br>oxybenzal-<br><i>p</i> -phenetidine |
|--|---|--|
|  |   |  |
| 1. <i>p</i> -Anisal- <i>p</i> -anisidine                     | 101.0   | 103.5  |
| 2. <i>p</i> -Anisal- <i>p</i> -toluidine                     | 39.0  | 39.0   |
| 3. <i>p</i> -Chlorobenzal-<br><i>p</i> -phenetidine          | 90.0  | 90.0   |
| 4. <i>p</i> -Dimethylamino-<br>benzal- <i>p</i> -phenetidine | 95.0  | 97.5   |
| 5. <i>p</i> -Dimethylamino-<br>benzal- <i>p</i> -anisidine   | 51.8  | 54.0   |
| 6. <i>p</i> -Chlorobenzal-<br><i>p</i> -toluidine            | ..  | ..   |
| 7. <i>p</i> -Dimethylamino-<br>benzal- <i>p</i> -toluidine   | ..  | ..   |
| 8. <i>p</i> -Ethoxybenzal-<br><i>p</i> -toluidine            | 76.0  | 76.0   |
| 9. <i>p</i> -Anisal- <i>p</i> -phenetidine                   | 121.0   | 121.0  |
| 10. <i>p</i> -Ethoxybenzal-<br><i>p</i> -anisidine           | 120.8   | 120.8  |

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### ION ASSOCIATION OF BIDIValent ELECTROLYTES IN DIOXANE-WATER MIXTURES AT 35° C

THE alternative theories on viscosity<sup>1-3</sup>, give different values of B-coefficient of the dissociated electrolyte. The viscosities<sup>4,5</sup> of MgSO<sub>4</sub>, ZnSO<sub>4</sub> and NiSO<sub>4</sub> in dioxane-water mixtures at 35° C are discussed in the present note in the light of theories in vogue.

The plot of  $\eta_r - 1/\sqrt{C}$  vs.  $\sqrt{C}$  is not linear, which shows that the Jones-Dole equation is inadequate. However the data are satisfactorily represented by the modified form of the Jones-Dole equation<sup>1</sup>.

$$\eta_r = 1 + A\sqrt{C} + BC^x$$

where 'x' is an empirical constant which varies from electrolyte to electrolyte but is near about unity as in Jones-Dole equation<sup>3</sup>.

Definite indications have been obtained from conductance measurements<sup>5-7</sup> that dissociation is incomplete. Hence the Davies-Malpass treatment<sup>2</sup> should be more suitable since it was formulated specifically to account for the effect of ion association. The treatment is as follows:

For symmetrical electrolytes of degree of dissociation 'α', Jones-Dole equation may be written as,

$$(\eta_r - 1 - A\sqrt{C})/C = \alpha(B_i - B_{ip}) + B_{ip}$$

where  $B_i$  and  $B_{ip}$  are the 'B' coefficients for the dissociated solute, and for the ion pair respectively. So a plot of  $(\eta_r - 1 - A\sqrt{C})/C$  against 'α' should have a slope of  $(B_i - B_{ip})$  and an intercept at  $\alpha = 0$ , of  $B_{ip}$ . However it is unnecessary to determine the slope, since the value of  $B_i$  may be found directly as the intercept at  $\alpha = 1$ .

In the present work, the value of 'α', the degree of dissociation was determined by successive approximations. Values of the activity coefficients of the ions were calculated by means the extended Debye-Huckel expression and 'α' is obtained by solving the equation:

$$K = \frac{a^2 c}{1 - \alpha} f^2$$

The activity coefficient of the undissociated salt was assumed to be unity. Extrapolation of the plot of the function  $(\eta_r - 1 - A\sqrt{C})/C$  against 'α' at  $\alpha = 0$  and  $\alpha = 1$  yielded the value of  $B_{ip}$  and  $B_i$ . The values thus obtained along with the 'B' values obtained from the modified equation have been recorded in Table I, from which it is seen that the 'B' value progressively increases as the dioxane content in the medium increases, suggesting that in the solution an orientation of the dioxane, with respect to the ion takes place, and this exhibits an increase in the viscosity and hence an increase in 'B' value.

From Table I, it is seen that the 'B' values of  $SO_4^{--}$  when paired with  $Mg^{++}$ ,  $Zn^{++}$  and  $Ni^{++}$  is not the same. The value of the  $SO_4^{--}$  when paired with  $Mg^{++}$  is least and when paired with  $Ni^{++}$  it is maximum (although they are almost same in water). This suggests that the value also depends on the cations with which the anion is paired and  $Mg^{++}$  has got the strongest structure breaking influence and the  $Ni^{++}$  the least and  $Zn^{++}$  is in between

these two. Hence it is concluded that the structure breaking influence increases with the increase in charge density of the ions.

TABLE I

| Electrolyte | Wt %<br>of<br>dioxane | Different B-values |      |      |
|-------------|-----------------------|--------------------|------|------|
|             |                       | I                  | II   | III  |
| $MgSO_4$    | 0                     | 0.59               | 0.68 | 0.52 |
|             | 10                    | 0.69               | 0.72 | 0.61 |
|             | 20                    | 0.76               | 0.78 | 0.66 |
| $ZnSO_4$    | 0                     | 0.58               | 0.70 | 0.50 |
|             | 10                    | 0.76               | 0.83 | 0.69 |
|             | 20                    | 0.81               | 0.89 | 0.71 |
| $NiSO_4$    | 0                     | 0.59               | 0.73 | 0.54 |
|             | 10                    | 0.70               | 0.81 | 0.67 |
|             | 20                    | 0.79               | 0.87 | 0.68 |

I—B from modified Jones-Dole equation.

II— $B_{ip}$  from Davies-Malpass equation.

III— $B_i$  from Davies-Malpass equation.

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#### CHEMICAL COMPONENTS OF INDIAN MEDICINAL PLANTS (*THESPIA POPULNEA* WOOD AND *CALYOPTERIS FLORIBUNDA* FLOWERS)

In continuation with our studies concerning the chemical constituents of medicinal plants growing in South India, we have now studied in detail the heartwood of *Thespesia populnea* and the flowers of *Calycopteris floribunda*. The results of these studies are briefly reported in the present communication. The medicinal uses of these plants are recorded elsewhere<sup>1</sup>.

The heartwood shavings of *Thespesia populnea* (440 g.) were repeatedly extracted with petroleum ether (60–80°). The extract upon concentration yielded a pale yellow mass which could be crystallised from chloroform-petroleum ether mixture. The yellow crystalline substance (m.p. 182–84°;  $(\alpha)_D^{25} + 432^\circ$ ; c, 0.15  $CHCl_3$ ) was found to be single entity by P. C. and T. L. C. Colour reac-

tions with concentrated sulphuric acid (red), ferric chloride (green) and sodium hydroxide (violet) all resembled those of gossypol closely. Confirmation of the identity was obtained by an examination of its U.V. spectrum ( $\lambda_{\text{m.x}}^{\text{EtOH}}$  237 and 378 nm), by the preparation of its acetate (m.p. 248–50°) and anil (300° dec). An authentic sample obtained from the bark of the same plant compared well with the specimen in hand.

*Calycopteris floribunda* is a dense shrub and its leaves are known to have laxative and anthelmintic properties and the juice from the twigs is used against malarial fevers, dysentery and diarrhoea. No chemical examination seems to have been reported on the flowers of this plant. The air-dried flowers (1 kg.) were extracted with methanol. On concentration a greenish-black residue which, when extracted with benzene, yielded the benzene-soluble calycoperin (P.C.; T.L.C. and mixed melting point). The benzene-insoluble portion was found to be essentially inorganic in nature.

The alcoholic filtrate yielded calycoperin when extracted with ether. The alcoholic mother liquor when allowed to stand for a few days in an ice-chest deposited a solid which was found to be a mixture of two components by paper chromatography. The fast-moving compound ( $R_f$  0.97, B : A : W 4 : 1 : 5) was found to be identical with calycoperin while the slow-moving compound ( $R_f$  0.85; B : A : W 4 : 1 : 5) was identical with quercetin. Their identity was further confirmed by isolation of each one of them by preparative paper chromatography and comparison with authentic samples.

The mother liquor after removal of all the aglycones was found to be glycosidic in nature. (Molisch test) and as no glycoside could be isolated, the extract was straight away hydrolysed with 7% sulphuric acid. From the hydrolysate an aglycone could be isolated which was found to be identical with quercetin.

Further study of the glycoside is in progress.

Our thanks are due to the University Grants Commission, New Delhi, and the Madurai University, for the award of a Research Scholarship to one of us (S.M.K.).

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# STUDY OF THE UNSAPONIFIABLE MATTER FROM THE ROOT OIL OF MIMOSA RUBICAU LIS

*Mimosa rubicaulis* (N.O. Leguminosae) is commonly planted in India and the powdered root is used when a patient, due to weakness, vomits his food. The medicinal value of the plant has been described by Kirtikar and Basu<sup>1</sup>.

The dried and powdered root (5 kg) was extracted with petroleum ether and the solvent distilled off at reduced pressure when a brownish coloured oil was obtained. It was saponified with 0.5 N alcoholic KOH and the unsaponifiable matter was then extracted with solvent ether. The unsaponifiable matter, a mixture of at least four constituents as revealed by TLC, was chromatographed over neutral alumina (Brockmann) with different solvents in increasing order of polarity.

Petroleum ether eluate furnished, after crystallisation from  $\text{CHCl}_3$ : MeOH, friedelin (100 mg), m.p. 260–62°,  $[\alpha]_D^{25} - 24^\circ$  ( $\text{CHCl}_3$ ) (Lit<sup>2</sup>. m.p. 262–63°,  $[\alpha]_D^{25} - 22.45^\circ$ ). Finally it was identified as friedelin by a comparison (mixed m.p. and I.R.) with an authentic sample. The IR spectrum showed a band at  $1715\text{ cm}^{-1}$  (six membered ring ketone). The mass spectrum exhibited peaks at  $m/e$  426 ( $M^+$ ), 411, 341, 302, 246, 213 and 205, suggesting its identity with friedelin<sup>3</sup>. Petroleum ether : benzene (1 : 1) eluate afforded  $\beta$ -amyrin (90 mg), m.p. 196–97°,  $[\alpha]_D^{25} + 85^\circ$  ( $\text{CHCl}_3$ ), identical (mixed m.p. and IR) with an authentic specimen (Lit<sup>4</sup>. m.p. 197–99°,  $[\alpha]_D^{25} + 88^\circ$ ). It gave L.B. test and Noller's reaction and an orange yellow colour with TNM. Acetylation with  $\text{Ac}_2\text{O}$  in pyridine produced an acetate, m.p. 236–37°,  $[\alpha]_D^{25} + 81^\circ$ . The mass spectrum, in addition to the molecular ion peak at  $m/e$  426 ( $M^+$ ), showed prominent peaks at  $m/e$  411, 218, 207, 205, 189, 149, 133 and 203 (base peak), indicating its identity with  $\beta$ -amyrin. The benzene eluate gave  $\beta$ -sitosterol (50 mg), m.p. 134–35°,  $[\alpha]_D^{25} - 36^\circ$  ( $\text{CHCl}_3$ ), identical (mixed m.p. and IR) with an authentic sample (Lit<sup>4</sup>. m.p. 134–36°,  $[\alpha]_D^{25} - 37^\circ$ ). The mass spectrum of the sterol exhibited principal fragment ions at  $m/e$  414 ( $M^+$  base ion peak), 399, 396, 273, 255 and 213 which are in accordance to the fragmentation pattern of  $\beta$ -sitosterol<sup>5</sup>. The petroleum used had b.p. 60–80°.

The authors are grateful to Prof. T. R. Govindachari, CIBA, for recording IR and mass spectra of the compounds and to University Grants Commission, New Delhi, for providing the financial assistance.

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### EFFECT OF CADMIUM CHLORIDE ON THE PROTEIN AND NUCLEIC ACID CONTENTS OF TESTES AND LIVER OF RAT

It has been observed<sup>1</sup> that the administration of cadmium chloride results in the depletion of ascorbic acid not only in the injected testis but also in the contralateral organ and the liver of albino rats. This led to the supposition that other biochemical components of the testes and liver might be influenced by cadmium chloride treatment.

The present report concerns the changes associated with protein and nucleic acids.

Albino rats (weighing between 80–115 g.) maintained at room temperature ( $30 \pm 2^\circ \text{C}$ ) on a diet of bread, milk and Bengal gram and water *ad libitum*, were divided into experimental and control groups. The experimental group of rats received a single dose of cadmium chloride (0.25 mg/100 g body weight) in their right testes. The contralateral testes were injected with an equal volume of sterilized distilled water. A group of rats of the same weight range served as the control which did not receive any treatment. After seven days, rats of both the groups were killed by the dislocation of neck. The testes and liver were prepared for protein and nucleic acid estimation in the same sample of tissue as described earlier<sup>2</sup>.

Results (Table I) indicate that except for the relative protein content (mg/g. wet wt.) in right testis, RNA/DNA ratio and absolute DNA content (mg/whole organ) of liver, all other values tend to decrease in the liver and testes of cadmium chloride treated animals.

TABLE I

*Effect of cadmium chloride on the protein and nucleic acid contents of testes and liver of rat*  
(3 rats per group. Mean  $\pm$  St. error)

|               | mg<br>Protein/<br>g.wet. wt.<br>tissue | %<br>change | gm. Protein/<br>organ<br>wet wt. | %<br>change | RNA/DNA<br>( $\mu\text{g}/\text{mg}$<br>Protein) | %<br>change | mg RNA/<br>organ<br>wet wt. | %<br>change | mg DNA/<br>organ<br>wet wt. | %<br>change |
|---------------|--|-------------|----------------------------------|-------------|--|-------------|-----------------------------|-------------|-----------------------------|-------------|
| Right testis: |  |             |                                  |             |  |             |                             |             |                             |             |
| Control       | 138 $\pm$ 12                           |             | 0.102<br>$\pm$ 0.032             |             | 0.66<br>$\pm$ 0.08                               |             | 2.82<br>$\pm$ 0.94          |             | 3.97<br>$\pm$ 0.87          |             |
| Experimental  | 154 $\pm$ 11                           | +11%        | 0.096<br>$\pm$ 0.029             | -6%         | 0.38<br>$\pm$ 0.13                               | -42%        | 1.01<br>$\pm$ 0.21          | -64%        | 3.36<br>$\pm$ 1.08          | -15%        |
| Left testis:  |  |             |                                  |             |  |             |                             |             |                             |             |
| Control       | 147 $\pm$ 4                            |             | 0.130<br>$\pm$ 0.002             |             | 0.84<br>$\pm$ 0.03                               |             | 3.50<br>$\pm$ 0.19          |             | 4.14<br>$\pm$ 0.30          |             |
| Experimental  | 131 $\pm$ 2                            | -11%        | 0.081<br>$\pm$ 0.017             | -38%        | 0.38<br>$\pm$ 0.18                               | -55%        | 2.21<br>$\pm$ 0.87          | -37%        | 3.93<br>$\pm$ 0.65          | -5%         |
| Liver:        |  |             |                                  |             |  |             |                             |             |                             |             |
| Control       | 440 $\pm$ 58                           |             | 2.270<br>$\pm$ 0.501             |             | 1.56<br>$\pm$ 0.40                               |             | 49.12<br>$\pm$ 1.86         |             | 35.52<br>$\pm$ 8.12         |             |
| Experimental  | 258 $\pm$ 32                           | -41%        | 1.263<br>$\pm$ 0.261             | -44%        | 2.11<br>$\pm$ 0.81                               | +35%        | 42.00<br>$\pm$ 8.91         | -14%        | 41.61<br>$\pm$ 29.81        | +17%        |

It seems that cadmium chloride influences the protein and nucleic acid syntheses of testes and liver of treated animals. Such effect of cadmium chloride is not only confined to the injected organ but it extends to contralateral testis and the liver also.

It may be that these changes are secondary to ischaemia caused by cadmium chloride treatment leading to less supply of metabolites required for the syntheses. The possible influence of cadmium chloride on the enzyme systems, connected with the syntheses and degradation of protein and nucleic acid also cannot be ruled out.

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ON A NEW AVIAN NEMATODE, *ASCARIDIA LUCKNOWENSIS* SP. NOV. FROM A CROW-PHEASANT, *CENTROPUS SINENSIS* (STEPHEN) FROM LUCKNOW

Four male and four female specimens of the genus *Ascaridia* were collected from the intestine of a Crow-Pheasant, *Centropus sinensis* (Stephen) from Lucknow. These represent a new species and are designated *Ascaridia lucknowensis* sp. nov. The nematodes were fixed in AFA (Alcohol-formaline, acetic acid) and later transferred to glycerine alcohol for clearing.

*Ascaridia lucknowensis* sp. nov. (Figs. 1-3)

Description

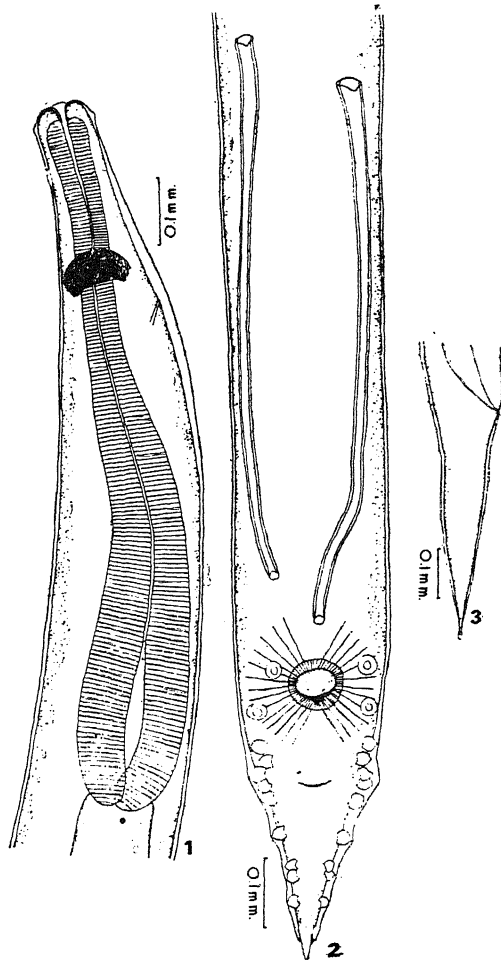
Body medium sized, slender (Fig. 1) tapering posteriorly. Lips well developed. Oesophagus club-shaped. Cuticle finely striated.

**Male** : Tail conical. Caudal alae feebly developed. Preanal sucker with cuticularized rim. Eleven pairs of sessile caudal papillae (Fig. 2) with four pairs preanal, two pairs adanal and five pairs postanal. Spicules subequal and similar with distal end rounded. Gubernaculum absent.

**Female** : Tail conical (Fig. 3). Vulva post-equatorial. Uterine branches opposed.

The new form differs from all the known forms of the genus *Ascaridia* Dujardin, 1845 except *A. trilabium* (v. Linstow, 1904) Railliet and Henry, 1914 and *A. anseris* Schwartz, 1925 in having spicules with distal end rounded. It differs from both these species in

the number and arrangement of caudal papillae. The new form further differs from *A. trilabium* in having subequal spicules instead of equal. Accordingly it is regarded as a new species with the specific name, *Ascaridia lucknowensis* sp. nov.



FIGS. 1-3. *Ascaridia lucknowensis* sp. nov. Fig. 1. Anterior end of male. Lateral view. Fig. 2. Posterior end of male. Ventral view. Fig. 3. Female tail. Lateral view.

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# RECORD OF *ANCISTROSYLLIS PARVA* DAY, 1963 (POLYCHAETA, PILARGIDAE) FROM THE NORTH-EAST COAST OF INDIA

WHILE engaged in the studies on *systematics* and *ecology* of the littoral polychaetes of Bhimilipatnam backwaters (Visakhapatnam) an interesting pilargid polychaete was observed in the samples. It has been identified as *Ancistrosyllis parva* which was erected by Day<sup>1</sup>. The area under investigation is mainly composed of fine mud mixed with fine sand rich in organic debris, where the salinity ranges from 15‰ to 40‰ and oxygen from 2 ml/L to 7.5 ml/L. The present communication records the occurrence of *Ancistrosyllis parva* for the first time from the peninsular India.

The specimens of *Ancistrosyllis parva* obtained in the present collections agreed in their characters with the type specimens. Day has given the number of papillae may vary from 10–12. The presently reported specimens have 12 marginal papillae (Fig. 1 A).

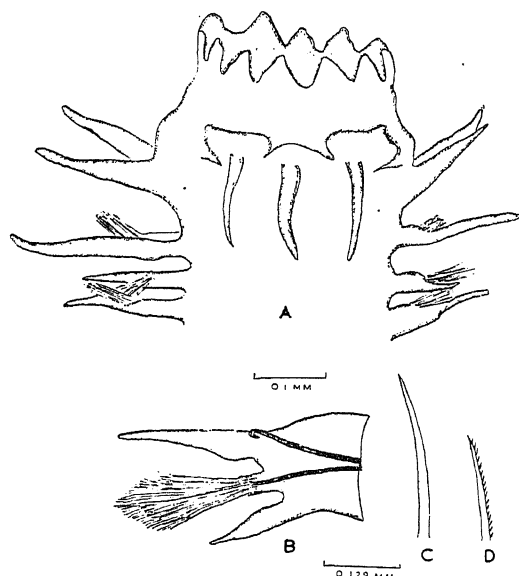


FIG. 1. *Ancistrosyllis parva*. A, Anterior end; B, Tenth foot; C, Long central neuroseta; D, Short outer neuroseta.

*Ancistrosyllis parva* was known earlier only from the type locality, i.e., West coast of South Africa (Natal and Cape) and recorded as endemic to the fauna of South Africa. The present record extends its distribution into the North-East Coast of India in the Northern Indian Ocean. Further intensive systematic exploration in the West coast of India and other geographical regions bordering the Indian ocean may bring to light the occurrence of *Ancistrosyllis parva* in those regions.

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September 13, 1975.

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## TAXONOMIC POSITION OF FLATFISH, *BRACHIRUS COMMERSONI* (LACEP.) AND *B. ALBOMACULATUS* (KAUP) (FAMILY: SOLEIDAE)

RECENTLY while examining specimens of *Brachirus commersoni* and *B. albomaculatus* from west coast of India, I noticed some characters which do not correspond to those of the genus *Brachirus*. Day<sup>1</sup> referred these species to the genus *Synaptura* Cantor but Norman<sup>2</sup> while considering *Synaptura* a junior synonym of *Brachirus* Swainson placed them under the latter. The generic position of these species is doubtful as they show following differences from *Brachirus orientalis* (Bl. and Schn.) which is the type species of the genus and also from the remaining Indian species of *Brachirus*.

|                                    | <i>B. com-<br/>mersoni</i> | <i>B. albo-<br/>maculatus</i> | Remain-<br>ing<br><i>Brachi-<br/>rus</i> spp. |
|------------------------------------|----------------------------|-------------------------------|---|
| 1. Body shape                      | Elongate                   | Elongate                      | Ovate   |
| 2. Bony projection<br>on the snout | Present                    | Present                       | Absent  |
| 3. Lateral-line scales             | 160                        | 155                           | 63–74   |

In Soleidae the ethmoid is produced into an elongated, flat and curved process which extends forwards beyond the vomer (Chabanaud<sup>3</sup>, Yazdani<sup>4</sup>). The bony projection on the snout of *B. commersoni* and *B. albomaculatus* on examination is found to be the projection of ethmoid process.

Chabanaud<sup>3</sup> gave figures of crania of some genera of Soleidae (*Solea*, *Brachirus* and *Zebrias*). The position of ethmoid process in these genera is clearly different from each other and also from that of *B. commersoni* and *B. albomaculatus*. In *Solea* and *Brachirus* the ethmoid process is curved towards the right or ocular side but in *Zebrias* it is curved towards the left or blind side. In all these genera, however, the ethmoid process remains hidden inside the snout. In *B. commersoni*

and *B. albomaculatus* the ethmoid process resembles that of *Brachirus* but its curvature is so much pronounced that it comes out as a bony prominence on the ocular side of snout.

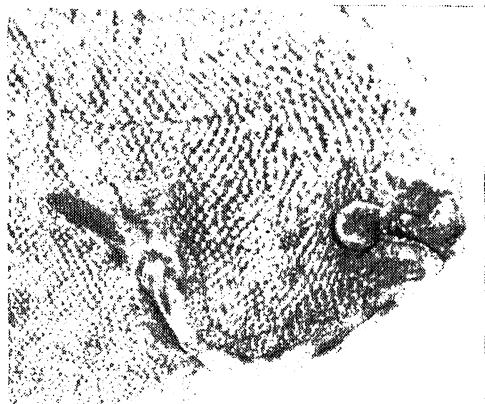


FIG. 1. Head of *Brachirus albomaculatus* (Kaup) showing bony projection on the snout.

In view of the differences observed above it would seem unjustified to place these species under *Brachirus*, although amongst Indian genera of Soleidae they show greater affinities with that genus. It seems, therefore, likely that *B. commersoni* and *B. albomaculatus* may either belong to a known genus from other waters or they represent an altogether new genus. In either case the examination of representatives of other genera of Soleidae becomes essential. Further work on these lines would, therefore, be desirable.

I am thankful to Dr. B. K. Tikader, Deputy Director, for facilities and to Dr. M. Babu Rao, Zoologist, for useful discussion.

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#### ON THE ACTIVITY OF CERTAIN SPIDER PREDATORS AGAINST STORED GRAIN INSECT PESTS

SINCE the stray record by Tsuteui (1937) who observed a spider, *Theridion tepidariourum* (Cambridge), preying upon the almond moth, *Ephestia cautella* (Walker), there have been very few studies

on the activity of spiders against stored grain insect pests. Recently, Battu *et al.* (1975) studied the predation of the larvae of *Trogoderma granarium* Everts by eight species of spiders. The present study has been extended to explore the use of some of these spiders against insects other than *T. granarium*, viz., *Sitophilus oryzae* L., *Rhizopertha dominica* F., *Tribolium castaneum* Herbst and *Alphitobius* sp.

The adults and juveniles of the various spiders were collected from the rural wheat stores in Ludhiana District during the months of July–August 1974. The spiders were starved for 1–3 days (Table I) and liberated in glass jars containing crushed wheat grains. The mouths of these jars were covered with muslin. Adults and/or larvae of *S. oryzae*, *R. dominica*, *T. castaneum* and *Alphitobius* sp. were offered separately to the starved spiders. Observations on the mortality of host insects were made after every 24 hours and the data are presented in Table I.

All the six spider species, viz., *Artema atlanta* Walck. (Pholcidae), *Marpissa* sp. (Salticidae), *Oecobus* spp. and *O. putus* Cambridge (Oecobiidae), *Selenops agumbensis* Tikader (Selenopidae), and *Uloborus danolius* Tikader (Uloboridae) were found to be actively preying upon the host insects. It was found that excepting *A. atlanta*, all other spiders caught hold of the insect hosts with the help of first pair of legs and pedipalpi. They pierced the preys with cheliceral fangs and continued sucking the body juices intermittingly till the prey was partially and/or completely squeezed. *A. atlanta* constructed webs of whitish silken threads. Cheliceral fangs were used for sucking the body fluids and the prey was reduced to completely squeezed carcass.

The larvae of *T. castaneum* seemed to be the most preferred host by all the spider predators tested. The average time taken to consume a good size *T. castaneum* larva was less than half a day for all the spider species. *Marpissa* sp. seemed to be the most efficient as it took the least time (0.05 day) to consume a single *T. castaneum* larva. Adults of *O. putus*, *Marpissa* sp. and *S. agumbensis* also took, on an average, less than half a day to consume a single *S. oryzae* adult, *Alphitobius* sp. larva and *R. dominica* adult respectively.

It may, thus, be seen that the spider predators can play an important role in regulating the populations of major stored grain insect pests. All the spider species tested have been found to be very efficient predators of *T. castaneum*. *A. atlanta* and *S. agumbensis* are equally good in preying upon *S. oryzae* and *R. dominica* respectively.

TABLE I

Efficacy of some spider predators against various stored grain insect pests

| Spider predators            |                                  | Insect hosts tested           |                |                     |   | Average time (days) taken to consume a single host (Y/X) |
|-----------------------------|----------------------------------|-------------------------------|----------------|---------------------|---|--|
| Name and Stage              | Average starvation period (days) | Name and stage                | Number offered | Number predated (X) | Average time (days) taken for predation (Y) |  |
| 1                           | 2                                | 3                             | 4              | 5                   | 6   | 7  |
| <i>A. atlanta</i> juveniles | 1.0                              | <i>T. castaneum</i> larvae    | 10             | 10                  | 0.90  | 0.09   |
|                             | 2.0                              | <i>R. dominica</i> adults     | 5              | 5                   | 4.25  | 0.85   |
|                             | 3.0                              | <i>S. oryzae</i> adults       | 5              | 5                   | 1.05  | 0.21   |
| <i>Marpissa</i> sp. adults  | 1.0                              | <i>Alphitobius</i> sp. adults | 5              | 5                   | 1.95  | 0.39   |
|                             | 1.5                              | <i>T. castaneum</i> larvae    | 15             | 15                  | 0.75  | 0.05   |
|                             | 2.0                              | <i>T. castaneum</i> adults    | 10             | 9                   | 10.62                                       | 1.18   |
| <i>Oecobus</i> sp. adults   | 2.5                              | <i>S. oryzae</i> adults       | 5              | 5                   | 8.50  | 1.70   |
|                             | 1.0                              | <i>R. dominica</i> adults     | 5              | 4                   | 11.20                                       | 2.80   |
|                             | 1.0                              | <i>S. oryzae</i> adults       | 5              | 5                   | 12.00                                       | 2.40   |
| <i>O. putres</i> adults     | 1.0                              | <i>T. castaneum</i> adults    | 10             | 9                   | 8.01  | 0.89   |
|                             | 1.0                              | <i>T. castaneum</i> larvae    | 10             | 10                  | 3.00  | 0.30   |
|                             | 1.0                              | <i>Alphitobius</i> sp. larvae | 5              | 5                   | 3.75  | 0.75   |
| <i>S. agumbensis</i> adults | 2.0                              | <i>T. castaneum</i> larvae    | 10             | 10                  | 2.00  | 0.20   |
|                             | 1.0                              | <i>R. dominica</i> adults     | 20             | 13                  | 4.94  | 0.38   |
| <i>U. danoli</i> adults     | 1.0                              | <i>T. castaneum</i> adults    | 20             | 3                   | 5.01  | 1.67   |
|                             | 1.5                              | <i>T. castaneum</i> larvae    | 6              | 6                   | 3.90  | 0.65   |

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#### CHROMOSOME NUMBER OF *DANAUS* *PLEXIPPUS* (LEPIDOPTERA: DANAIDAE)

EVEN though *Danaus* constitutes the largest genus of the family Danaidae with 17 representative species in India, a few of which are cosmopolitan in distribution, our knowledge of the chromosome cytology of this genus is very meagre at present. Chromosome numbers of only five species in this genus are known till to date. This note reports the chromosome number of one more species of *Danaus*, viz., *D. plexippus* (L.).

Acetic-orcein squash preparations of the testes of *D. plexippus* collected near the Postgraduate Centre, without pre-fixation show 30 bivalents in Metaphase I (Fig. 1). Little variation in size among the bivalents was noted. In Metaphase I stages, while some of the bivalents were still having non-terminalised chiasmata, a few other bivalents were almost separated into the homologues, being in contact only near their ends.

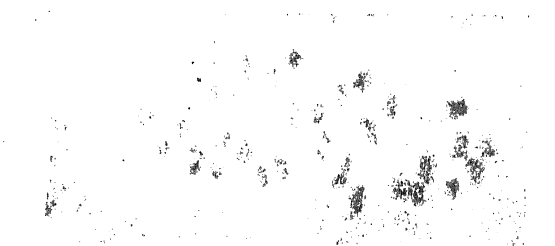


FIG. 1. Metaphase I chromosomes of *D. plexippus* (L.).

In the genus *Danaus*, the chromosome number has been reported so far, for the following species :

1. *D. chrysippus* L. (Srivastava and Gupta<sup>1</sup>, Gupta<sup>2</sup>, de Lesse and Condamin<sup>3</sup>,  $n = 30$ ) ; 2. *D. gilippus* Cr. (Maeki and Remington<sup>4</sup>,  $n = 29$ ) ;

3. *D. eresimus* Cr. (Maeki and Remington<sup>4</sup>,  $n = 30$ ); 4. *D. hamata septentrionis* Butler (Saitoh and Abe<sup>5</sup>,  $n = 31-36$ ); 5. *D. Limniace* Cr. (= *Tirumala limniace* of Saitoh and Abe<sup>5</sup>,  $n = 33$ ); *D. limniace limniace* (Maeki and Ae<sup>6</sup>,  $n = 37$ ) and *D. limniace petriverrana* (Bernardi and de Lesse<sup>7</sup>,  $n = 41-46$ ).

Variation in the chromosome number in different nuclei of the same specimen of *D. hamata septentrionis* was reported by Saitoh and Abe, whereas very wide variation in chromosome number in different subspecies of *D. limniace* from different geographical regions was reported by different workers, suggesting attempts at evolution of subspecies into species at chromosomal level. The chromosome number of *D. plexippus*, not only agrees with the number in two other species of *Danaus* but with that of the most common haploid number of Lepidoptera (Suomalainen<sup>8</sup>).

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## IMPROVING SEED GERMINATION WITH MIST

MANY crop plants pose problem in seed germination, the causes being physical and physiological. The freshly harvested seeds of many temperate crops like strawberry, grapes and many pome fruits are in a state of dormancy and unless they receive adequate chilling, the dormancy is not broken and seeds do not germinate. In strawberry, Iyer, Chacko and Subramanyam<sup>1</sup>, have shown that treatments of fresh seeds with Ethrel (2-chloro-ethane-phosphonic acid) at 5,000 ppm for 24 hours induced nearly 90% germination within four weeks and these findings have

further been corroborated by the studies of Wilson, Goodall and Reeves<sup>2</sup>. Further studies by Iyer and Subramanyam<sup>3</sup> have suggested that the promotion of germination with Ethrel might be due to the inhibition of the growth inhibitors that are present in the strawberry seeds. Since in a mist chamber, the seeds are continuously being exposed to mist and there is continuous washing of the seeds, it was logical to conclude that better germination could be obtained if seeds are sown in pots kept in mist chambers. The studies by Wilson *et al.*<sup>2</sup>, have given very promising results. The present study deals with the excellent results that have been obtained with a number of strawberry varieties.

## Materials and Methods

Seeds extracted from freshly harvested fruits of five varieties of strawberry, namely, Bangalore Local, Gorella, Robinson, Senga Sengana and Tioga were sown in seed pans on a medium containing equal quantities of fine sand and soil. Five such seed pans were kept in the mist chamber in the month of May, 1974 after the initial watering of the medium. In the mist chamber, the pots were exposed to intermittent sprays of mist for 20 seconds at an interval of every 3½ minutes. Equal number of pots with seeds sown were kept in one part of the chamber where they were not exposed to mist sprays but which were watered twice a day which acted as the control. Observations were made every day on the rate of germination.

## Results and Discussion

The extent of seeds that germinated within a period of one month is presented in Table I. The seeds that were kept in the mist chamber germinated much faster than the control and in different varieties, the success obtained ranged from 79.6 to 93.3% within the course of a month from sowing.

TABLE I  
Seed germination recorded within 30 days of sowing

| Variety         | Per cent germination |         |
|-----------------|----------------------|---------|
|                 | Mist Chamber         | Control |
| Bangalore Local | 93.3                 | 2.2     |
| Gorella         | 87.8                 | 3.1     |
| Robinson        | 86.4                 | 2.9     |
| Senga Sengana   | 79.6                 | 1.1     |
| Tioga           | 90.0                 | 1.4     |

In contrast to this, in the controls, the germination was only ranging from 1.1 to 3.1%. The growth of the seedlings was also found to be much faster in the mist chamber. The data taken on the vegetative growth of the seedlings after 3½ months

of sowing showed that in the mist chamber, the seedlings were nearly 15 cm in height, whereas, the few that germinated in the controls had attained only about 3 cm.

Another interesting observation was that irrespective of the parentage of the seeds, every seedling that was raised in the mist chamber produced large number of runner plants and hence vegetative propagation of even four month old seedlings could be possible. In the control plants, the seedling size was so small that none of the plants produced any runners within this period. The plants that were raised in the mist chamber reached transplantable stage much earlier.

Using the mist technique of seed germination, it was possible to assess the performance of hybrid seeds in a very short time. It was possible to obtain fruiting in the seedlings within a course of six months from seed sowing which is a great boon in the breeding programme of this crop. All other methods currently known take not less than a year.

The present study, therefore, has shown that germinating seeds in a mist chamber has manifold advantages. Firstly, it is not necessary to stratify the seeds for three months under cold conditions. Early and high germination can be obtained even with freshly extracted seeds using mist technique. Secondly, the seedlings raised in the mist chamber are fast growing and hence come to fruiting much earlier which facilitates earlier seedling evaluation and thirdly, every seedling produces abundant runners under mist and hence, it is possible to vegetatively propagate the selected seedling at a much faster rate.

The highly significant effect of mist on seed germination and seedling growth even in plants known to have inherent dormancy appears to be due to (i) the leaching of the germination inhibitors that are present in the seeds and (ii) permanent state of turbidity of seeds and seedlings in mist chamber. Strawberry fruits and seeds are known to have growth inhibitors (Rudnicki, Pieniazek and Pieniezek<sup>4</sup>; Varga<sup>5</sup>, and Negi and Singh<sup>6</sup>). Negi and Singh (1973) observed that when the seeds of strawberry were kept in running water for 4 days, nearly 27.5% seeds had sprouted in the bag itself. Further, when the washed seeds were treated with water extract obtained from unwashed seeds, the germination was significantly reduced, almost to the level of unwashed seeds. These observations lend support to the presence of some water soluble substance that inhibits germination.

This method can be applied successfully in a number of crops where good and fast germination cannot be obtained easily. We have tested this

method on the seeds of *Carica*, Pineapple and Guava and have obtained highly positive results. This method is now being extended to other crops also.

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#### THE GENETICS OF FIELD IMMUNITY TO BROWN RUST AND ITS INCORPORATION INTO COMMERCIAL VARIETIES OF WHEAT

KALYAN SONA, a widely adapted high yielding wheat (*Triticum aestivum* L.) cultivar, has become susceptible to the most prevalent races of brown rust (*Puccinia recondita* Rob. ex Desm.) (12, 77, 162, 162A, 104, 77) in India. A vigorous search for germplasm to provide sources of resistance is being carried out at different breeding centres. At the Punjab Agricultural University, Ludhiana, an exotic strain EC 93131 (Atlas-66) of wheat (*T. aestivum* L.) was detected to exhibit field immunity to all the above races of brown rust consistently for a number of years. This was true also at other locations in India (Stroike *et al.*, 1973). Therefore, it was planned to study the genetic nature of immunity of this line and explore the possibility of transferring this characteristic to Kalyan Sona.

The parents, F<sub>1</sub> (Kalyan Sona × EC 93131) and F<sub>2</sub> were space-planted in 1972-73 at the PAU experimental plots. The above lines along with the F<sub>3</sub> were also space-planted in a randomized complete block design with two replications in 1973-74. The infector rows, a mixture of Agra local and Lal Bahadur (*T. aestivum* L.) were planted all around and were inoculated with a mixture of the above-mentioned prevalent races in both the years. The individual plants were scored as immune (completely free of disease) or diseased (traces to 100% infection).

The  $F_1$  plants were as immune as EC 93131. It indicated that the gene for immunity was dominant over susceptibility. The plants in the  $F_2$  segregated in a 3 (immune) : 1 (diseased) ratio (Table I).

TABLE I

Segregation for field immunity to prevalent races of brown rust in various generations of the cross, Kalyan Sona  $\times$  EC 93131

| Generation  | Number of plants |     |                       |     | $\chi^2$ |
|---|------------------|-----|-----------------------|-----|----------|
|   | Observed         |     | Expected <sup>a</sup> |     |          |
|   | I                | D   | I                     | D   |          |
| Kalyan Sona   | ..               | All | ..                    | All | ..       |
| CC 93131  | All              | ..  | All                   | ..  | ..       |
| F <sub>1</sub>                                      | All              | ..  | All                   | ..  | ..       |
| F <sub>2</sub>                                      | 219              | 81  | 225                   | 75  | 0.64     |
| F <sub>3</sub> (random)                             | 155              | 95  | 156                   | 94  | 0.04     |
| F <sub>3</sub> (Susc. F <sub>2</sub> plants selfed) | 3 <sup>b</sup>   | 247 | ..                    | 250 | ..       |

<sup>a</sup> .. On the basis of segregation for one dominant gene for immunity

<sup>b</sup> = These plants were probably the escapes

I = Immune

D = Diseased

$\chi^2$  = (05,  $\infty$  df) = 3.84.

Thus it appeared that immunity is governed by one dominant gene. This observation was confirmed by the segregation in  $F_2$  generation. The plants in the  $F_3$  derived from randomly selected  $F_2$  individuals segregated in the ratio of 5 (immune) : 3 (diseased). This is as expected on the basis of one dominant gene for immunity. All the progeny of the diseased  $F_2$  plants was also diseased which supports the above conclusion.

An attempt was made to search for immune plants in the  $F_2$  and  $F_3$  populations which resembled Kalyan Sona. It appeared to be too early to recover such individuals. The probability of recovering such segregates will be high if the immunity gene is not associated with any undesirable character of the EC 93131 parent. This parent is relatively tall, late, has lax ears and its grains are soft and red.

A comparison for various agronomic characters of the immune and diseased plants would indicate if the immunity gene is associated with undesirable traits of EC 93131. Such a comparison could not be made for yield and 1000-grain weight because of the obvious difficulty of providing true checks for diseased plants in the segregating progenies. Therefore, two groups were compared only for height, days to earing and maturity. The data for these three characters indicated differences of a small

magnitude between the two groups (Table II) which, however, are not likely to be of practical importance. Therefore, the incorporation of the gene for immunity into the genotype of Kalyan Sona may not produce any adverse effect.

TABLE II

Data (mean) on height, heading date and maturity for the immune and diseased plants in the  $F_2$  and  $F_3$  populations

| Generation  | Reaction | Plant height (cm) | Heading date (days) <sup>1</sup> | Maturity date (days) <sup>1</sup> |
|-------------|----------|-------------------|----------------------------------|-----------------------------------|
| $F_2$       | D        | 106.11            | 111.85                           | ..                                |
|             | I        | 106.00            | 113.02                           | ..                                |
| L.S.D. (05) |          | 1.83              | 1.23                             | ..                                |
|             | $F_3$    |                   |                                  |                                   |
| $F_3$       | D        | 102.20            | 114.10                           | 152.80                            |
|             | I        | 102.86            | 112.42                           | 150.74                            |
| L.S.D. (05) |          | 1.80              | 0.92                             | 0.49                              |

<sup>1</sup> = Number of days after seedling.

A practical approach would be to initiate a back-cross programme to transfer the immunity gene of EC 93131 with Kalyan Sona as the recurrent parent. Some of the immune plants in  $F_3$  were closer to Kalyan Sona in characters like height and maturity. Use of these  $F_3$  plants as donors may reduce the number of backcrosses and the time required to restore the Kalyan Sona phenotype and yielding ability.

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#### AN APPROACH TOWARD PLANT TYPE CONCEPT IN SUGARCANE

AN attempt is made to study certain physiological parameters which can explain the yield differences among sugarcane cultivars. A consistently high yielding variety Co. 62175 was subjected to growth analysis by the method outlined by Watson (1952) and compared with the commercial check, Co 419 (Table I).

TABLE I

Certain Physiological parameters in Co 62175 and Co 419 during grand growth period, i.e., 14–28 weeks after planting

| Variety  | Yield<br>5 years<br>Av.<br>1968–73<br>(tons/ha) | Pol in Juice<br>at harvest<br>4 yrs. Av.<br>1968–72<br>(%) | CCS<br>2 yrs.<br>Av.<br>1968–70<br>(tons/ha) | Fibre<br>in cane<br>(%) | Leaf area<br>per clump<br>(dm <sup>2</sup> ) | NAR<br>(g/dm <sup>2</sup> /wk) | RGR<br>(g/g/wk) | CGR<br>(g/wk) | LAR<br>(dm <sup>2</sup> /g) |
|----------|---|--|--|-------------------------|--|--------------------------------|-----------------|---------------|-----------------------------|
| Co 62175 | 188   | 19.7   | 28.2   | 15.2                    | 140.0  | 0.63                           | 0.17            | 53            | 0.31                        |
| Co 419   | 149   | 20.4   | 22.8   | 20.1                    | 87.1   | 0.34                           | 0.09            | 21            | 0.29                        |

The experiment with 6–8 promising cane cultures was arranged in a randomised block design, with four replications in five seasons. The net plot size in all seasons varied from 72 m<sup>2</sup> to 90 m<sup>2</sup>. Cane yield and CCS tons/ha differed significantly with CV of the experiment less than 10%. Parameters such as leaf area (L), net assimilation rate (NAR), relative growth rate (RGR) and leaf area ratio (LAR) were the averages of two clumps from one replication. Light transmission ratio (LTR) is an average of ten fixed points in the crop canopy near the ridge at an approximate height of 30 cm. A prediction equation involving three yield attributes has been worked out.

Table 1 indicates that during grand growth period extending from 14–28 weeks after planting, Co 62175 maintained the superiority over Co 419 in respect of total leaf area/clump (L), NAR, CGR, RGR and LAR. It is worth noting that NAR, a measure of photosynthetic efficiency, was nearly double in Co 62175 compared to Co 419 (Table I). McLean *et al.* (1968) observed that variation in yield of sugarcane varieties can be caused both by variation in NAR and in LAI. Another significant factor noticed in Co 62175 was a linear relationship between LAI and CGR upto LAI 7.4 (Fig. 1) during grand growth period.

#### Plant type concept

Earlier studies aimed at finding out certain growth attributes in *Saccharum* clones<sup>1</sup> and yield components in sugarcane<sup>2,4</sup>. It is fairly established that the yield components in sugarcane are the stalk number, stalk diameter and stalk length and a negative relation exists between the stalk number and stalk diameter and between the stalk weight and stalk number<sup>2</sup>.

In our investigation, an attempt has been made to estimate the combined effect of three yield attributes on cane yield of two dozen cultivars by a multiple regression equation of the type:

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3$$

where  $x_1$  = stalk number,  $x_2$  = stalk length and  $x_3$  = weight/cane,  $a$ ,  $b_1$ ,  $b_2$ ,  $b_3$  are the constants.

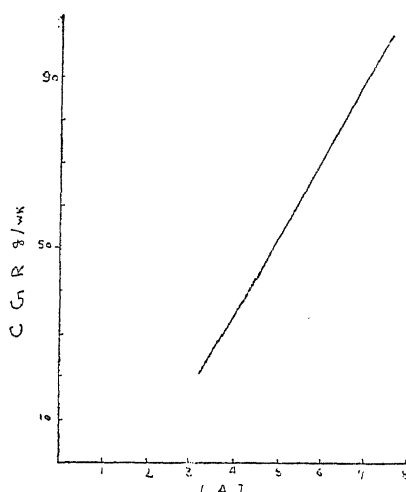


Fig. 1. Regression of crop growth rate (CGR) on leaf area index (LAI) in Co 62175 (14–28 weeks).  $Y = 18.4235x - 40.4476$  where  $Y =$  CGR g/wk,  $x =$  LAI std. error for slope,  $sb = 2.1558^*$ . Correlation coefficient,  $r = 0.95^*$ .

We obtained the relation as indicated in equation 1.

$$Y = 796.0247 + 0.7905x_1 + 11.5959x_2 + 3.9776x_3 \dots R^2 = 0.79^{**} \quad (1)$$

The stepwise regression indicated that stalk length, weight/cane and stalk population in that order contribute to the cane yield. Since stalk length and diameter are genetically controlled emphasis has to be placed in accommodating higher stalk density per unit area without reduction either in weight or length of cane.

It is suggested that clones with higher LTR values at grand growth period accommodate higher stalk density without adversely affecting the other yield attributes. LTR measures the light interception by the crop canopy and is given as  $I/I_0 = e^{-KL}$ , where  $I$  = light intensity below the crop canopy,  $I_0$  = light intensity above the crop canopy,  $L$  = leaf

\* Significant at 1% level.

\*\* Significant at 5% level.

TABLE II

Light transmission ratio, LTR ( $I/I_0 \times 100$ ) in some sugarcane cultivars at two different growth stages

| Sl. No. | Cultivar       | Growth stage I (weeks) | LTR % | Sl. No. | Cultivar       | Growth stage II (week) | LTR % | Remarks  |
|---------|----------------|------------------------|-------|---------|----------------|------------------------|-------|--|
| 1.      | B 37172        | 13-15                  | 27.0  | 1.      | Co 62175       | 16-18                  | 8.5   | Desirable plant types are B 37172, Co 62175, H. 2857, Q 49 in that order. Severe tiller mortality was observed in H. 2045 and 66 Co A-11 at this location. |
|         |                |                        |       | 2.      | H. 2857        | 16-18                  | 7.7   |  |
|         |                |                        |       | 3.      | Q 49           | 16-18                  | 7.3   |  |
| 2.      | Co 419 (Check) | 13-15                  | 23.0  | 4.      | Co 7112        | 16-18                  | 5.8   |  |
|         |                |                        |       | 5.      | 66 Co A-11     | 16-18                  | 5.4   |  |
| 3.      | H. 2045        | 13-15                  | 14.0  | 6.      | Co 419 (Check) | 16-18                  | 5.2   |  |

area index and  $K$  = extinction coefficient. LTR is measured as  $I/I_0 \times 100$ .

Table II is suggestive that the desirable plant types are B 37172, Co 62175, H 2857 and Q49 which have higher LTR values. Mention may be made of the varieties like B 37172 and Q49 which possess erect and thick leaves and can accommodate 2-3 times higher density (0.2-0.3 million/ha) than Co 419 (0.10-0.12 million/ha), without reducing either the length, girth or weight/cane. However information is lacking on crop extinction coefficient,  $K$ , of these genotypes.

The limited data presented on two varieties suggest that the desirable plant types should have higher values of LAI, CGR and NAR. Further, emphasis has to be placed in selection of clones with thick and erect leaves and which can accommodate higher stalk density, i.e., 0.2 to 0.3 million/ha without reduction in stalk length, stalk diameter and weight/cane and this is perhaps possible in cultivars with higher LTR and lower  $K$  values.

A replicated trial conducted at this station with six varieties confirms the trends herein reported.

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### INFLUENCE OF MORPHACTIN, MALEIC HYDRAZIDE AND CYCOCEL ON YIELD AND POST-HARVEST QUALITIES OF POTATO (*SOLANUM TUBEROSUM* L.)

THE production of tubers in potato (*Solanum tuberosum*) can be increased by an additional supply of nitrogen (Ghosh and Kanjaria<sup>1</sup>; Salter and Williams<sup>2</sup>) and by exogenous application of some plant growth substances (Smeltzer and Mackay<sup>3</sup>; Das and Prusty<sup>4</sup>; Shukla and Jauhari<sup>5</sup>). The present investigation was carried out during winter season of 1972-73 to determine the effects of morphactin (Methyl-2-chloro-9-hydroxyfluorene-9-carboxylate) cycocel (2 chloroethyl trimethyl ammonium chloride) and maleic hydrazide on yield size, quality and post-harvest changes during the storage of potato tubers (*Solanum tuberosum* L. cv. Kufri Alankar).

The cut pieces of potato tubers were kept at 1, 10, 100 ppm in each of the growth substance for 10 min. The cut pieces dipped in water for ten min served as control. The pieces were sown in the pots containing soil mixed with farm-yard manure. The pots were arranged in a randomised block of ten plants and replicated thrice. Moisture content of the tuber was determined by drying at 85° C for 48 hours. The starch and ascorbic acid were estimated according to AOAC methods<sup>6</sup>. The potato tubers were kept at 5° C for 100 days to study harvest modifications. Results are presented in Table I. The presowing application of morphactin significantly increased the production of tubers gradually with increase in concentration and CCC was also found to increase the production of tubers, but MH decreased the production of tubers (Table I). The maximum large tuber were produced by CCC followed by morphactin, minimum larger tuber were produced by MH over control (Table I). Morphactin increased dry weight % whereas dry weight % was decreased by MH and CCC (Table I)



TABLE I

Effect of morphactin, maleic hydrazide and cycocel on reduction and post-harvest qualities of potato (*Solanum tuberosum* L.)

| Observations                 | Control         | Morphactin       |                  |                  |                  | Maleic hydrazide |                  |                  |                  | Cycocel          |  |
|------------------------------|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|--|
|                              |                 | 1 ppm            | 10 ppm           | 100 ppm          | 1 ppm            | 10 ppm           | 100 ppm          | 1 ppm            | 10 ppm           | 100 ppm          |  |
| Yield of tuber/<br>plant (g) | 485.2<br>±23.7  | 501.7<br>±25.4   | 520.4<br>±26.1   | 563.5<br>±25.2   | 482.2<br>±22.7   | 454.7<br>±22.1   | 430.1<br>±21.8   | 509.8<br>±24.3   | 629.3<br>±28.8   | 670.8<br>±31.9   |  |
| Size of tuber                |                 |                  |                  |                  |                  |                  |                  |                  |                  |                  |  |
| Large                        | 33              | 39               | 41               | 43               | 25               | 27               | 27               | 71               | 75               | 78               |  |
| Medium                       | 31              | 34               | 30               | 31               | 39               | 37               | 38               | 20               | 17               | 12               |  |
| Small                        | 26              | 13               | 11               | 6                | 27               | 25               | 20               | 4                | 5                | 7                |  |
| Chats                        | 10              | 14               | 18               | 20               | 9                | 11               | 15               | 5                | 3                | 3                |  |
| Dry weight %                 | 21.5            | 23.2             | 23.7             | 24.0             | 19.3             | 19.0             | 19.1             | 17.7             | 17.4             | 18.1             |  |
| Moisture %                   | 78.5            | 76.8             | 76.3             | 76.0             | 82.1             | 80.7             | 80.9             | 82.3             | 82.6             | 82.6             |  |
| Starch %                     | 20.0            | 21.3             | 21.0             | 23.8             | 21.0             | 22.5             | 23.0             | 21.1             | 21.4             | 21.9             |  |
| Ascorbic acid<br>mg/100 g.   | 12.31<br>± 0.21 | 11.79*<br>± 0.34 | 11.72*<br>± 0.26 | 11.88*<br>± 0.31 | 11.87*<br>± 0.16 | 11.92*<br>± 0.27 | 11.95*<br>± 0.20 | 11.95*<br>± 0.25 | 11.91*<br>± 0.34 | 11.98*<br>± 0.22 |  |
| Post-harvest qualities       |                 |                  |                  |                  |                  |                  |                  |                  |                  |                  |  |
| Loss of weight of<br>tuber % | 18.5            | 11.9             | 11.6             | 10.2             | 17.9             | 17.8             | 17.2             | 44.7             | 44.9             | 44.4             |  |
| Rottage of tuber             | 5.8             | 23.5             | 20.0             | 18.2             | 22.9             | 22.4             | 22.0             | 38.1             | 38.4             | 37.0             |  |

\* Not Significant.

Morphactin and MH increased the starch contents while CCC reduced it (Table I). Morphactin, MH, and CCC had no significant effect on ascorbic acid content. CCC increased weight loss of tubers while morphactin and MH decreased it (Table I). The highest percentage of rootage was recorded in CCC treatments, MH slightly increased rootage and morphactin decreased it (Table I).

The observations of the present study clearly demonstrate that CCC increased the production. Similar observations were made by Das and Prusty. Morphactin also raised the production. Our findings do not agree with those observations reported previously for potato (Merck<sup>7</sup>). MH slightly decreased the yield of tuber. These observations are in accordance with those of reported previously (Das and Prusty). It is clear from the results that morphactin increased dry matter and starch contents. CCC decreased starch contents of tubers. The present findings can be explained simply by suggesting that the plant growth substances affect the yield, chemical composition and post-harvest qualities by modifying growth and development through metabolism in *S. tuberosum*.

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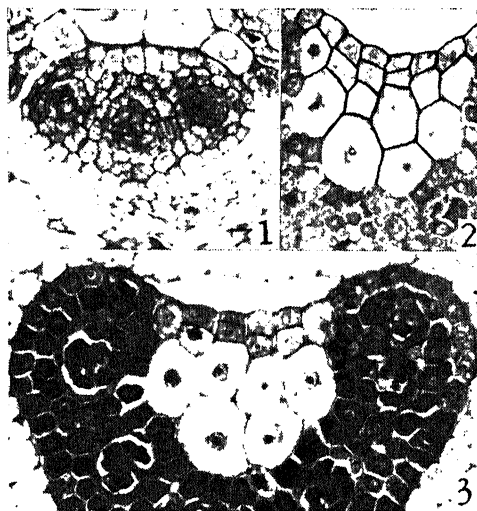
#### MULTIPLE EPIDERMIS OF *FIMBRISTYLIS* *SCHOENOIDES*

SOME species of *Fimbristylis* (Cyperaceae) have a colourless region of cells in the leaf-blade between the adaxial epidermis and chlorenchyma. Metcalfe<sup>1</sup> referred to this zone as adaxial hypodermis. Sharma and Mehra<sup>2</sup> provisionally called it multiple epidermis on the basis of suggestive alignment of its cells with those of mature epidermis. The

present study was made to investigate the nature of this hypodermis. No such information is available for the Cyperaceae.

The nuts of *F. schoenoides* Vahl were sown in petri dishes on wet blotting-paper. The plumules and basal portions of 1-leafed seedlings, enclosed within cotyledonary sheaths and situated above mesocotyl, were fixed in F.A.A. They were microtomed following the usual procedure.

In transection of very young leaf-blade, the protodermal cells on the adaxial side become more conspicuous than the rest (Fig. 1). They undergo 1-3 successive periclinal divisions (Fig. 2). After every division, it is the outer cell that divides further. The number of divisions decreases from the median to marginal cells (Fig. 2). The inner cells become remarkably large, with very conspicuous nuclei, and their radial alignment is disturbed (Fig. 3). Besides the adaxial group, the



FIGS. 1-3. Young epidermis in transections of leaf-blade of *Fimbristylis schoenoides*. Fig. 1. Uniseriate protoderm. Fig. 2. Adaxial protoderm after periclinal divisions. Fig. 3. Multiple adaxial epidermis. All figures,  $\times 280$ .

other protodermal cells that divide periclinally are the progenitors of subepidermal fiber strands, situated at the margins and on the abaxial side. The inner derivative of each such cell gives rise to a group of fiber initials. The outermost derivatives of protoderm mature into epidermis, but in the median region on the adaxial side 1-3 such cells, separated from one another divide periclinally once or twice. The resultant inner or median cell produces a group of fiber initials, while the outermost cell divides anticlinally giving rise to two epidermal cells (cf. Sharma and Mehra<sup>2</sup>). Under

certain conditions the adaxial protodermal cells may not divide periclinally, and mature into uniseriate epidermis of conspicuously large cells.

Ontogenetically, the adaxial hypodermis and all the subepidermal fiber strands are derived from protoderm. Thus the adaxial hypodermis represents the multiple epidermis.

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#### A NEW SPECIES OF THE GENUS *OCTOSPORA* FROM INDIA

THE *Octospora* Hedw. ex Gray is a valid and established genus<sup>1,2,5,6</sup>, consisting of more than forty species growing mainly on bare soil, soil among mosses, humus, rotten wood and leaves, with the exception of *O. ithacaensis* (Rehm) Khare<sup>4</sup> which is a weak parasite on *Marchantia polymorpha*. The genus is accepted here in a broad sense and two other genera *Inermisia* Rifai and *Koillabaea* Svrcek, previously segregated from the genus *Octospora*<sup>7,8</sup> have been reduced to the subgeneric level on account of the presence of intermediate forms, existing between the three genera<sup>3</sup>.

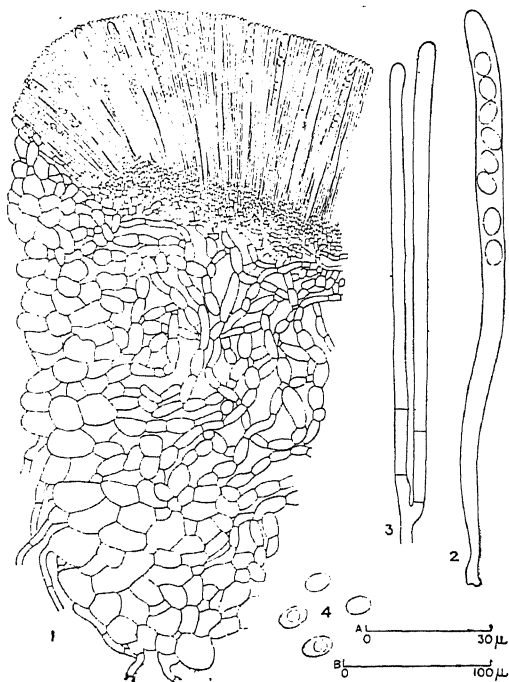
A single collection of this genus was made in the campus of Banaras Hindu University, India, in 1969. On comparison with the other species of the genus *Octospora* known so far, this was found to represent a new species. Its illustrated account is provided in the present note. Specimen studied has been deposited in the Banaras Hindu University, Plant Pathology Herbarium, abbreviated as BHUPP.

*Octospora luticola* Khare, sp. nov. (Figs. 1-5).

Apothecia ca. 3-3.5 cm., sessilia dispersa, sulphurea usque ad aurantia, applanata vel revoluta; asci octospori, ope jodi non-caerulescentes, brevicylindrici, 100-130  $\times$  5.5-7  $\mu$ ; ascospores minutae, ovoideo-ellipticae, glabrae, uniguttulatae, uniseriate, 5.5-7  $\times$  4-5  $\mu$ ; paraphyses simplices rectae tenues 1-2 septatae; excipulum medullosum tenuitunicatum, textura intricata constitutum, in partibus aliquibus cellulas intercalarias inflatas efformante; excipulum ectale texture angularis, cellulis sat magnis composition; hyphae tomenti ex excipulo ectali enatae, hyaline septatae. Typus BHUPP 1265 in solo.

Apothecia (Fig. 5) scattered, 3-3.5 cm in diam., sessile, flat tending to curved downward; hymenium

sulphur yellow to orange; externally lighter, pubescent. In section (Fig. 1): hymenium 100–125  $\mu$  thick; subhymenium 30–38  $\mu$  thick, of short angular to elongated cells; medullary excipulum 125–600  $\mu$  thick, consisting of textura intricata, hyphae 7–15  $\mu$  wide, thin walled, septate, at places forming intercalary inflated cells; ectal excipulum 75–150  $\mu$  thick, consisting of 'textura angularis', cells 15–40  $\mu$  in diam; tomentum hyphae 10–15  $\mu$  wide, septate, colourless, more or less entangled, drawn out from the end cells of the ectal excipulum; asci (Fig. 2) J-, 8-spored, cylindric, 100–130  $\times$  5.5–7  $\mu$ ;



FIGS. 1–4. Camera lucida drawings of *Octospora luticola*. Fig. 1. V.S. of a part of an apothecium. Fig. 2. A complete ascus. Fig. 3. A branched paraphysis. Fig. 4. Ascospores. Scale line A for Figs. 2, 3, 4 and B for Fig. 1.



FIG. 5. Apothecia of *Octospora luticola*,  $\times$  1.3.

ascospores (Fig. 4) small, hyaline, ovoid-elliptical, smooth, uniguttulate, uniseriate, straightly or obliquely arranged, 5.5–7  $\times$  4–5  $\mu$ ; paraphyses (Fig. 3) simple, straight, 1–2 septate, slender, almost equal to the asci and 3–4  $\mu$  thick throughout.

*Habitat*.—On loamy soil.

*Etymology*.—Latin 'lutum' = mud or loam + 'colus' = to live, referring to the habitat being loamy soil.

*Holotype*.—BHUPP 1265, Leg. K. B. Khare, September 18, 1969.

*Type Locality*.—Old Botanical Garden, Banaras Hindu University, Varanasi.

The large sized, repand apothecia with sulphur yellow colour and uniguttulate, small ascospores are distinguishing characters of the present species.

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#### AN EMS INDUCED 'ZEBRA' MUTANT IN RICE

THE first reference of 'Zebra' striping was given by Ramiah and Ramanujam<sup>1</sup> who obtained seedlings with alternate transverse bands of white and green on the leaf blades in a pure line of rice. Subsequently similar 'zebra' mutants were reported in x-ray treated population of rice. In the present communication the phenotypic pattern and the inheritance of one zebra mutant obtained from an American cultivated variety of rice, Saturn is described.

The zebra mutant was obtained in the  $M_2$  population of EMS (0.4% aqueous solution, for six hours

proceeded by a presoaking of dehusked seeds for 18 hours in distilled water) treated population. The frequency of this mutation was 7 in a population of 13195 (0.053%)  $M_2$  plants. The fact that such mutation has been known to occur in natural as well as mutagen treated population is indicative that the gene(s) causing zebra phenotype might be sensitive to mutation as has been reported in the case of 'Grandpa' mutant in barley<sup>3</sup>.

**Phenotypic Expression.**—The zebra character manifested shortly after germination. The seedling after germination became slender and pale yellow. The clear expression of zebra striping started from the first true leaf where it exhibited alternate transverse white and green bands. Generally 2-3 white bands occurred in first and second leaves. The later leaves showed more white bands and a maximum of seven white bands per leaf were found in healthy seedlings. However, some plants showed variation in the distribution of white and green bands. In some cases the basal position of the leaves remained white and only the distant half or one-third of the leaves were green and hence it could be mistaken as *virido-alba* as per the classification of Gustafsson<sup>4</sup>. But careful examination reveals the difference. In the case of *Virido-alba* the basal portion of the leaves were white and the green pigments gradually became intense towards the tip whereas in the case of zebra mutant the two bands were clearly demarcated giving a characteristic zebra pattern (Fig. 1).

After the transplantation of the seedlings, the banding pattern became inconspicuous in subsequently formed leaves. The cross bands gradually became more pronounced from the fifth leaf stage and remained upto seventh leaf stage. Thereafter the green and white bands coalesce and the clarity of white bands became blurred. Most of the secondary tillers at this stage appeared white with fine green streaks throughout the tiller including the flag leaf, the leaf sheath, the peduncle, the panicles and the spikelets. At this stage the plant produced large number of tillers and the dwarf habit became conspicuous. It exhibits chlorophyll deficiency almost similar to 'Grandpa' mutant of barley<sup>3</sup>.

Unlike the zebra necrotic genes reported in maize<sup>5</sup> and sorghum<sup>6</sup>, the white bands in this zebra mutant did not develop necrosis but remained healthy and produced normal pollen and healthy seeds. Probably the green portions provided enough leaf area for normal photosynthetic function of the plants.

**Inheritance Pattern.**—Inheritance of zebra character was determined from the study of  $M_3$  population as well as from the  $F_2$  population of

crosses between zebra and normal (saturn) and its reciprocal and from the first back cross progenies of (zebra  $\times$  normal)  $\times$  zebra which indicated that the character was controlled by a single recessive gene (Table I) apparently having no maternal

TABLE I

Segregation ratios for normal : zebra phenotypes

| Progenies                  | Nor-<br>mal | Zebra | Total | Ratio | 2     | p value<br>between |
|----------------------------|-------------|-------|-------|-------|-------|--------------------|
| $M_3$ — 1                  | 244         | 78    | 322   | 3:1   | 0.102 | 0.80-0.70          |
| 2                          | 346         | 113   | 459   | 3:1   | 0.018 | 0.90-0.80          |
| 3                          | 287         | 99    | 386   | 3:1   | 0.085 | 0.80-0.70          |
| 4                          | 236         | 77    | 313   | 3:1   | 0.016 | 0.90-0.80          |
| 5                          | 177         | 57    | 234   | 3:1   | 0.050 | 0.90-0.80          |
| Zebra $\times$<br>Normal   | 400         | 136   | 536   | 3:1   | 0.380 | 0.70-0.50          |
| Normal $\times$<br>Zebra   | 386         | 127   | 513   | 3:1   | 0.012 | 0.95-0.90          |
| (Zebra $\times$<br>Normal) | 177         | 168   | 345   | 1:1   | 0.234 | 0.70-0.50          |
| Zebra) — BC.1              |             |       |       |       |       |                    |

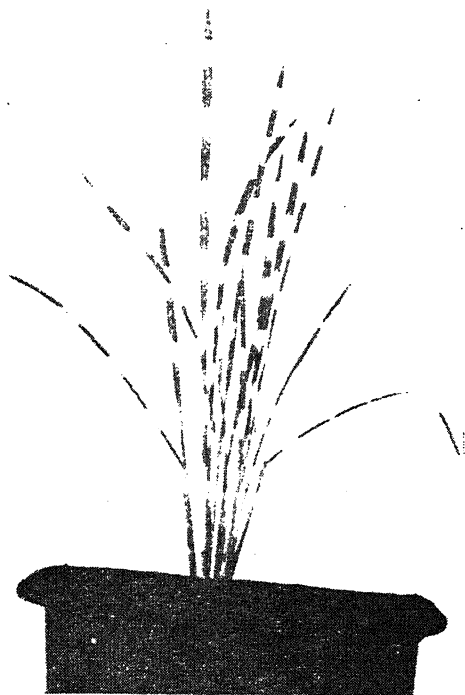


FIG. 1. Photograph of a 65 days old zebra seedlings.

effect and the zebra locus might also be linked with dwarfing habit.

Some of the mutant progenies as well as  $F_2$  and  $F_3$  progenies showed a high degree of chlorophyll deficiency at seedling stage, with leaves completely white or rarely with only one or two green transverse bands. Some (4 to 5%) of these seedlings died within 15 to 25 days after germination and this may, however be accounted for, a recessive lethal deficiency of zebra mutants, a situation very similar to that observed in many of the induced mutants which are sub-vital<sup>7</sup>.

From the present findings it has not been possible to outline any definite explanation to account for the development of zebra pattern in leaves. However it is noticed that under ambient temperature and diurnal fluctuation of light, the zebra pattern of leaves is expressed better whereas change in these factor leads to irregularity in the expression. This suggests that possibly under the influence of temperature and light fluctuation in day and night the zebra gene results in such localized chlorophyll development of leaves.

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#### A CYTOGENETICAL NOTE ON DOUBLE-GRAINED RICE (*ORYZA SATIVA* L. VAR. *PLENA* PRAIN)

*Oryza sativa* L. var. *plena* Prain is popularly known as double-grained rice, cultivated in localised areas in West Bengal as wet season crop. Sikder<sup>4</sup> studied its floral morphology in detail. Two types of grains are found in this variety, long and short. The present note deals with the karyotype and meiotic

studies on plants, grown from these two types of grains and compared with the normal single-grained variety.

The long and short and the normal grains were de-husked and germinated in Petridishes on moist filter paper. Suitable root tips were selected, pretreated with aqueous solution of Aesculine<sup>1,2</sup>, at 8° C for 3 hours and fixed in Propionic acid alcohol 1 : 2 for 30 min. Propiono-orcin staining technique was followed. For meiotic study, Propiono-carmin staining technique was followed after fixing the spikelets in propionic acid alcohol 1 : 2 for 24 hours.

Somatic chromosome number, karyotype configuration and haploid number of these three types of plants are given in Tables I and II.

TABLE I  
Somatic chromosome number and karyotype configuration of 3 plants grown from double-grained rice

| Source of the taxon | Somatic chromosome number | Types observed |    |    | Ranges between short and long chromosome in $\mu$ |
|---------------------|---------------------------|----------------|----|----|---|
|                     |                           | A              | B  | C  |   |
| Long grain ..       | $2n = 26$                 | 4              | 8  | 14 | 0.8-2.2   |
| Short grain ..      | $2n = 22$                 | 4              | 10 | 8  | 0.1-2.0   |
| Normal grain ..     | $2n = 24$                 | 4              | 6  | 14 | 0.8-2.5   |

Type A: Medium size chromosome with secondary constriction.

Type B: Medium size chromosome with median to submedian primary constriction.

Type C: Short size chromosome with median to submedian primary constriction.

TABLE II  
Haploid number of 3 plants grown from double-grained rice

| Source of the taxon | Total number of cells observed | 12 II | 13 II | 11 II | Percentage                               |
|---------------------|--------------------------------|-------|-------|-------|--|
| Long grain          | 65                             | 15    | 45    | 5     | 12 II, 23<br>13 II, 69.3<br>11 II, 7.3   |
| Short grain         | 65                             | 18    | Nil   | 47    | 12 II, 27.7<br>13 II, Nil<br>11 II, 72.3 |
| Normal grain        | 65                             | 55    | 5     | 5     | 12 II, 84.6<br>13 II, 7.7<br>11 II, 7.7  |

The normal chromosome number of rice<sup>2</sup> (*Oryza sativa* L.) is  $2n = 24$ . In the present investigation, two different chromosome numbers have been observed from the plants developed from the two types of grains obtained from the double-grained variety. In the plants developed from long grains, the chromosome number was found to be  $2n = 26$  and at meiosis, 69% cells had  $n = 13$  chromosomes. In the plants developed from short grains, the somatic chromosome number was  $2n = 22$  and 72% of the cells showed  $n = 11$  bivalents in meiotic study. Thus, the two types of plants developed from seeds of the same double-grained variety, selected from bulk showed somatic chromosome number of 26 and 22 which are unusual for *Oryza sativa* L. as the parent variety had the normal chromosome number  $2n = 24$ .

It is remarkable that in nearly all the two types of plants derived from the double-grained rice, the karyotype difference is not pronounced (Figs. 1 a, 3 a and 5 a) and no distinction can be made between

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### CHROMOSOMES PAIRING IN AN AUTOTRIPLOID RICE

IDENTIFICATION of autotriploids in a short duration, photoinensitive rice variety which can be grown in both dry and wet seasons, will be useful for the development of aneuploid lines. The meiotic behaviour of such an autotriploid, isolated from  $M_2$  population of the variety *Sona* (IET. 1991) treated with 0.2% aqueous solution of EMS for 6 hours preceded by a presoaking in distilled water for a period of 6 hours is reported here. The frequency of occurrence of this autotriploid was 3 (0.05%) out of 5437  $M_2$  population.

This triploid exhibited gigas habit, having long bold spikelets, increased plant height, short and robust awn similar to the earlier reports of spontaneous autotriploids<sup>3,7,9,11,12,15</sup>. The pollen and spikelet sterility observed in this triploid were 94.3% and 99.7% respectively, compared to the earlier reports (from 76.3% to 88.4% in the case of pollen and from 96.6% to 99.4% in the case of spikelet)<sup>11-13</sup>.







Cytological study of meiosis in autotriploids has been attempted by many earlier workers<sup>1-5,7,10,14</sup>, who reported various types of meiotic abnormalities regarding the number and type of multivalents. However, the maximum number of trivalents were observed in the autotriploids so far was 10 per PMC<sup>12</sup>. Cytological analysis of PMCs at metaphase and anaphase I stages in the autotriploids reported here revealed the occurrence of very high frequency of trivalents followed by bivalents and univalents in lesser frequency. The maximum number of PMCs showed 11 trivalents whereas the frequency of trivalents ranged from 4-12 (Fig. 1) and the bivalents and univalents varied from 0-6 per PMC (Table I). The occurrence of equal proportion of bivalents and univalents indicated that they might have arisen due to the failure of formation of trivalents or to early terminalization of chiasmata.



FIGS. 1-5 a. Fig. 1, 1 a and 2. Somatic metaphase, idiogram and diakinesis of the plant, grown from long grain showing  $2n = 26$  and  $n = 13$  chromosomes. Figs. 3, 3 a and 4. Somatic metaphase, idiogram and meiotic metaphase of the plant grown from short grain showing  $2n = 22$  and 11 chromosomes. Figs. 5 and 5 a. Somatic metaphase and idiogram of the plants grown from the normal grain.

one taxon and another on the basis of the karyotype. The presence of  $2n = 26$  and  $2n = 22$  chromosomes, indicates the evidence of aneuploidy at an intravarietal level. It is likely that in the natural population of the double-grained variety, spontaneous occurrence of triploids and monosomics is quite possible. These may give rise in later generations to heterogeneous plants giving tetrasomic ( $2n + 2$ ) and nullisomic ( $2n - 2$ ) plants which may have survived in natural selection.

TABLE I  
Chromosome configurations and the frequency in a  
triploid rice induced by EAFS

| No. and configura-<br>tion of<br>trivalents  | Fre-<br>quency<br>of PMCs | No. of<br>associa-<br>tions | Range | Mode |
|--|---------------------------|-----------------------------|-------|------|
| 1.  | 145                       | 894                         | 1-10  | 5    |
| 2.   | 94                        | 142                         | 0-4   | 1    |
| 3.  | 77                        | 104                         | 0-4   | 0    |
| 4.   | 103                       | 200                         | 0-5   | 1    |
| 5.  | 23                        | 38                          | 0-5   | 0    |
| 6.  | 59                        | 40                          | 0-4   | 0    |
| Σ Trivalents   | 145                       | 1418                        | 1-12  | 11   |
| Σ Bivalents  | 145                       | 310                         | 0-6   | 1    |
| Σ Univalents   | 145                       | 262                         | 0-6   | 1    |
| Σ Quadrivalents  | 145                       | 21                          | 0-2   | 0    |

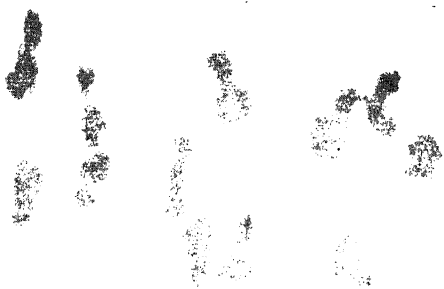


FIG. 1. PMC at metaphase I showing a maximum of 12 trivalents (9 frying pan, 1 chain and 2 Y shaped).

Critical analysis of frequency and types of multi-valent association has not been done in autotriploids. In the present study, trivalents of frying pan type occurred in a maximum frequency followed by the concentric ring and chain types. The least frequent was the triangle type. The extremely low recovery of such a configuration might be either due to chance association, or one of the constituent chromosomes in such a trivalent would have two identical arms. However, the latter possibility is less expected in the present case, since out of a total 1418 trivalents scored in 145 PMCs at MI only 23 PMCs showed the triangle type. Although the maximum frequency of trivalents per cell was 11, maximum number of frying pan shaped was six per PMC. The orientation of the trivalents and the disjunction at anaphase I lead to the formation of aneuploid gametes. In some PMCs (19) however, 1 to 2 quadrivalents were observed. Since most

of them were chain types, they might have occurred probably due to chance or loose association of Is and IIIs, although the segmental homology of the constituent chromosome leading to quadrivalent formation might not be ruled out.

Anaphase I exceedingly normal, the disjunction being 17-19 in maximum cases and only 7% of PMCs at anaphase I showed 22-14 and out of 26 PMCs studied at anaphase I, 12-24 or 13-23 disjunction could not be observed indicating that  $n + 1$  pollen formation might be as such less. Further at telophase I, 1-8 laggards were observed. In one case, 3 IIs exhibited non-disjunction and in another a trivalent, behaved likewise. The lagging chromosome often got themselves distributed to the poles. Mitotic division of the univalents also occurred in a few cells. Probably at metaphase II the laggards, i.e., both univalents and half univalents were not included and were eliminated as restitution nuclei.

The second meiotic division was analysed in a limited number of cells. At anaphase II, it was observed that many of the lagging univalents were not included in the normal chromosome movement during the second meiotic division. The univalents or the half univalents which were not included in nucleus at the end of meiosis could be found as micro-nuclei again at the tetrad stage. Thus, the apparent similarity in distribution of chromosomes at A II and A I, is in agreement with the observation of Munzing<sup>8</sup> and Lange and Wagenvoort<sup>6</sup> in *Solanum*.

The authors are grateful to Dr. S. Y. Padmanabhan, Director, for providing the facilities and constant encouragement.

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## SHORT SCIENTIFIC NOTES

### Foot-rot of Oats Caused by *Sclerotium rolfsii*

Oats (*Avena sativa*), grown in experimental plots, were severely diseased in the flag leaf stage at the Agricultural College Farm, Hebbal, in February 1975. A close examination of the affected plants indicated foot-rot symptoms, marked by dark-brown discolouration at the collar region, covered by a white cottony mycelial growth. On uprooting such plants, the stem gave away breaking easily at ground level. In advanced conditions, numerous sclerotia were seen on the collar region. Isolations from the infected portions yielded *Sclerotium rolfsii* Sacc.

For testing pathogenicity, the fungus was grown on cornmeal sand medium for a week and mixed into the top layer of sterilized soil filled in 6" clay pots. Surface sterilized oat seeds were then sown. Typical root-rot symptoms were observed 30–35 days after sowing. The organism was reisolated from such infected plants.

Although this pathogen has been reported on several hosts from India, there is no record of this pathogen on oats. This constitutes a first record of *S. rolfsii* on oats from India. The culture has been deposited in the culture collection of Department of Plant Pathology, U.A.S., Bangalore (No. 124).

Grateful thanks are due to Dr. H. C. Govindu for providing facilities.

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### A New Record of Safflower Wilt Incited by *Fusarium solani*

Singh *et al.* (1975) reported two wilts of safflower incited by *Fusarium* species from Varanasi region. A third disease incited by another species of *Fusarium* was observed recently in Varanasi region.

The disease is characterised by the abnormal growth including short internodes and at times plants appear bushy. The main tap root undergoes rotting and cluster of adventitious secondary roots develop to keep the plant alive for some time.

The lamina on both sides of the midrib are not even, leaves become sickle shaped. Sometimes the leaves become as narrow as a tape; veins and midrib become indistinguishable. Numerous dot

like brown spots appear on the upper surface of the cotyledonary leaves. Sometimes 30% inhibition of seed germination has been recorded.

A species of *Fusarium* was isolated from diseased roots. The mycelium is cottony or spreading type on PDA, floccose or submerged on Richard's medium. Colour changes from white to orange finally to grey. Rate of growth 1.01 cm to 2 cm per day on PDA at 32° C. Microconidia abundant, 3-septate, 14.8–37.0  $\times$  5.5–7.4  $\mu$ . Chlamydospores terminal or intercalary sometimes in long chains, smooth walled. The pathogen has been identified as *Fusarium solani* (Most) Sacc. The identification has been confirmed by the Commonwealth Mycological Institute, Kew (U.K.) (IMI-186540). Pathogenicity test was carried out in pots. Soil of the pot (soil : sand : FYM–3 : 1 : 1) was infested with the fungus previously grown on sand maize meal medium at the rate of 1.5 gm of inoculum per 100 gm of soil. Seven days after infestation, surface sterilized seed were sown. Equal number of unfested pots served as control. Typical symptoms were observed even at the cotyledonary stage while the control plants remained healthy, thus, confirming the pathogenicity of *F. solani* on safflower. This is the first record of *F. solani* inciting wilt of safflower.

Thanks are due to Dr. C. Booth, for confirming the identification of the pathogen.

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### Treatment of Amphistomiasis in Sheep

Amphistomiasis is one of the most serious parasitic diseases of the sheep which takes a heavy toll every year (Alwar, 1959). Acute amphistomiasis is fairly widespread in Bihar and occurs from October to March, after rains where potential snail vector *Indoplanorbis* exists. This note assess the efficacy of combined anthelmintic treatment.

The affected sheep were showing typical symptoms of the disease, anoraxia, bottle-jaw, and foetid diarrhoea, and were passing immature amphi-



stomes in their loose faeces. Post-mortem examination of 5 animals revealed numerous immature and mature amphistomes in the duodenum, abomasum and rumen assignable to *Cotylophoron* and *Gastrothylax* spp.

From this flock, 30 animals showing similar typical symptoms of the disease were divided into 3 equal groups. Group 'A' was given 1 ml carbon tetrachloride (C.T.C.), with 3 ml liquid paraffin subcutaneously at the thigh region along with 5 gm of Hexachloroethane orally. Group 'B' was given C.T.C. as above with 100 mg Hexachlorophene orally. Group 'C' was given 1 ml C.T.C. orally with rice gruel after copper sulphate swabbing.

After a week there was marked improvement in the general condition of animals of group 'A' and 'B' with only 2 and 1 deaths respectively. After 15 days all faecal samples from these groups were found negative for either amphistome eggs or their immature stages and the sheep recovered completely. Animals of group 'C' showed aggravated diarrhoea probably due to irritant action of the drug on the abomasal mucosa. Eight sheep died within a week and only 2 recovered.

These results suggest that carbon tetrachloride alone *per os* is not very effective but combination of anthelmintic like Hexachloroethane or Hexachlorophene orally with C.T.C. subcutaneously proves more effective in natural outbreak of amphistomiasis in sheep. This supports the work of Mitterpak (1958) on *Facioliasis* of sheep.

Disease Investigation

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### Diseases of Fruits from Haryana. I. A New Fruit Rot of *Zizyphus mauritiana* Lamk.

A disease of Ber fruit (*Zizyphus mauritiana* Lamk.) was observed and collected during March–April 1975 from the orchard of Haryana Agricultural University. The infected fruits were characterised by small, slightly depressed, dark-brown spots near the stem end. The erumpent pycnidia developed on these spots. Lesions were noticed on

the ripe fruits. The lesions were irregular in shape measuring 15–25 mm in diameter.

The fungus was isolated on Czapek's medium and the cultural study of the fungus was done at 25°. The pathogenicity was proved by putting the spore suspension on the fresh and healthy fruits after slight injury. Infection was established after 72 hours of inoculating the fungus on the healthy fruits and same types of lesions were formed.

The morphology of the fungus under study resembles that of Sphaeropsidaceae genus *Phoma* described so far. It differs from other reported species in morphology and parasitism. It also differs from *Phoma zizyphi* Pat. in having smaller pycnidia and larger conidia. Therefore, a new species, viz., *Phoma hissarensis* is being proposed to accommodate this fungus.

*Phoma hissarensis* spec. nov.

Coloniae in agaro 'Czapeks' primo ablae et floccosae tum pallide brunneae vel brunneae. Pycnidia evolvuntur in culture, nemerosa, fusce brunnea. Pycnidia globosa vel subglobosa, ostiolata, erumpentia, 107. 10 × 92.82 (85.68–128.52 × 71.40–114.24) μ in diam. Conidiophora simplicia, hyalina, Conidia unicellularia, hyalina, ovals Vel ellipticae 3.4–10.2 × 3.4 μ.

Colony on Czapek's agar white and flucose then turned into light brown to brown in colour. Numerous dark brown pycnidia form in culture. Pycnidia globose to subglobose, ostiolate, erumpent, measuring 107.10 × 92.82 (85.68–128.52 × 71.40–114.24) μ in diameter. Conidiophore simple, hyaline. Conidia unicellular, hyaline oval to elliptical, measuring 3.4–10.2 × 3.4 μ.

Varietal reaction of *Zizyphus* fruits to the above described fungus revealed that out of five varieties employed Seo-Bahadurgarhia was highly susceptible and it was followed by sonahari No. 5, Kaithi, Banarasi Karaka and Umran.

The culture is being deposited in Plant Pathology Laboratory, HAU Hissar (PPHAU 67), Herb. Crypt. India. Orient IARI, New Delhi and CMI, Kew, England.

The authors are grateful to Dr. B. S. Chundawat, Head of the Department, for providing laboratory facilities.

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## REVIEWS AND NOTICES OF BOOKS

**Physiological Aspects of Dryland Farming.**  
Edited by U. S. Gupta. (Oxford and IBH Publishing Co., New Delhi), 1975. Pp. xv + 391.  
Price : Rs. 80-00.

This book edited by Dr. U. S. Gupta, Plant Physiologist, Haryana Agricultural University, Hissar, India, contains nine review articles contributed by various authors. In the article on physiological principles of dryland crop production, Dr. Arnon of Settlement Study Centre, Rehovot has dealt with the interrelationships among climatic factors, evapotranspiration and crop yield, and management practices for improving the water supply available to the crop, and its efficient use. The topics of drought injury and resistance of crop plants by Dr. Larson of University of Missouri, the effect of humidity on crop production by Dr. O'Leary of University of Arizona and photorespiration in relation to crop yield by Dr. Goldsworthy of Imperial College of Science and Technology, London, have covered various aspects of drought stress in plants, related physiological processes and their effect on crop yields. The relationships of humidity and photorespiration on crop yields show interesting avenues for further research.

The role of mulches in dryland agriculture by Dr. Unger, Soil Scientist of USDA, Texas, deals with the effect of mulches on soil moisture conservation and, other soil environment and plant factors. The role of antitranspirants in arid agriculture presented by Dr. Davenport and Dr. Hagan of University of California, contains the effect of the use of anti-transpirants on transpiration and photosynthesis processes. While use of mulches for high value crops is possible, the use of antitranspirants, in general, has been found to curtail both transpiration and photosynthesis in plants. The topic on root patterns in crops as related to water and nutrient uptake dealt by Dr. Hurd and Dr. Spratt of Canada Department of Agriculture has brought out the possibility of increased crop yields with practices that favour root growth. Wind effects and their amelioration in crop production by Dr. Sturrock of Department of Scientific and Industrial Research, New Zealand and Heat Unit concept of crop maturity by Dr. Iwata of Tohoku National Agricultural Research Station, Japan are the other topics covered in this book.

This book is very useful for teachers and research workers in plant physiology, plant breeding

and agronomy as a reference book. The effort made by Dr. U. S. Gupta, in editing this book by compiling the research papers of high standard is commendable. The quality of printing and get up are good.

G. V. HAVANAGI.

**The Organic Chemistry of Tellurium.** By K. J. Irgolic. (Gordon and Breach Science Publishers, New York), 1975. Pp. xiv + 452. Price £12.80.

The stated purpose of this book is to completely present the field of Organic Chemistry of tellurium. The author has admirably achieved this in sixteen chapters starting with an account on structure and properties of elemental tellurium and concluding with an appropriate chapter on Biology of organic tellurium compounds. The organic compounds of tellurium have been treated in a systematic fashion, dealing with (a) Methods of introducing tellurium in organic molecules, (b) Compounds containing carbon-tellurium single bond, (c) Carbon-tellurium moiety, (d) Tri and tetra-organyl tellurium compounds, (e) Heterocyclic tellurium compounds and (f) Tellurium containing polymers.

It is pleasing to see a chapter on Nomenclature of Organic tellurium compounds and a valuable chapter on physicochemical investigations on organic tellurium compounds. This latter chapter presents useful data on infrared, ultra-violet and visible, nuclear magnetic resonance, mass spectral and other structural studies so far carried out in this type of compounds. A noteworthy feature being the appendix which describes the patents taken on organic tellurium compounds. The patents are classified according to the country, compound, reference and claim. This will be useful for those who have interest in application oriented research.

Each chapter is complemented with tables describing the methods of preparation and physical properties of compounds and neat figures depicting the reaction schemes. These will be very useful for a synthetic chemist and also for those who wish to venture into this field. It is heartening to see a section on coordination compounds having tellurium as donor.

This book gives not only a comprehensive account on the organic tellurium compounds but also indicates a good deal which remains to be done. It represents a good beginning for future

growth of research in this field. The author has done a creditable job of presenting the scattered information in a systematic, logical, and lucid form.

This book may be expected as a standard reference work in the organic chemistry of tellurium and should be available in all chemistry Libraries.

V. KRISHNAN.

**Laboratory Manual of Plant Pathology.** By V. N. Pathak. (Oxford and IBH Publishing, Co., New Delhi), 1974. Pp. ix + 212. Price Rs. 16.50.

There are 2 parts and in part 1 a general idea is given on handling glassware and equipment, use of microscope, acquaintance with plant pathogens, their isolation and purification, reproduction, collection and preservation, staining, physiology, chemical control and heat therapy. These are covered in the form of a series of exercises, 30 in all.

In the second part which relates to examination of plant diseases, a number of them are mentioned. The entire book is in the form of a laboratory manual and since most of the well-known diseases affecting crop plants, vegetables and fruits are discussed, the book will serve a useful purpose for students of agriculture.

K. SUBRAMANYAM.

**The Biology of Cancer.** By Armin C. Braun. Addison-Wesley Publishing Company, Massachusetts, U.S.A.), 1974. Pp. xi + 169.

This book is primarily intended for beginners who have a background knowledge in the basic biological sciences and who are interested in gaining insight into our understanding of the cancer problem. An attempt is made to identify the essential biological concepts that underlie the tumorous state and to critically evaluate the premises upon which prevailing thought in the field of experimental oncology is based.

In addition to being a medical problem of greatest urgency, cancer represents one of the most fundamental and challenging areas for study in the basic biological sciences. The experimental oncologist will have to explain as to why cancer cells divide persistently and in an unrestrained manner in their hosts and why such cells invade underlying normal tissues and metastasize to distant sites, while the growth of all normal cells is precisely regulated.

The existing knowledge pertaining to dynamic problems are subjected to a critical analyses and many facets of the 'Biology of Cancer' are lucidly presented in six chapters of this volume.

After a brief general introduction covering broadly the distinguishing characteristics of tumour cells, the causes of cancer and the role of hereditary factors in carcinogenesis, the author discusses at length "The Development of autonomy", "Somatic mutations", "Addition of new genetic informations", "The epigenetic changes" and "Biological approaches to the control of cancer" in the succeeding chapters.

M. SIRSI.

**Insecticides of the Future.** Edited by Martin Jacobson. (Marcel Dekker, Inc., New York), 1975. Pp. v + 93. Price : \$ 9.50.

In many countries approaches to insect pest control are now being re-oriented to decelerate, if not altogether halt, the ecological imbalances arising from the widespread and often indiscriminate use of organic insecticides. New insecticides are available and the problem of resistance has appeared at a most opportune moment. This book presents the present possibilities of developing rational insect control with the least disruption of the ecological balance. The collection of articles written by leading authorities and first published in *Letters*.

The editor explains the background of the subject in a short introduction, which is followed by five articles dealing, respectively, with the parasitoids and predators in biological control, insects, pathogenic micro-organisms as agents of insect suppression by manipulating natural immunity, as well as synthetic sex pheromones, sex attractants, and the development and possible applications of morphogenetic agents (chemicals which interfere with the growth and development of insects) in insect control. While each of these methods can be employed by itself one or more of them will also easily fit into any scheme of integrated control of pests.

Each article is an excellent review covering the author's own published and unpublished work and also the significant contributions of many other scientists, which are listed at the end. A cumulative author index is also provided.

T. SANKARAN.

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